

Nuclear Energy in Europe

ALL over Europe, governments and industrial companies seem to be kicking their heels, waiting to see what will happen next. Last week in Brussels, five European companies (Agip Nucleare SpA, Belgonucleaire SA, Interatom GmbH, Kraftwerk Union AG and the Nuclear Power Group Limited) threw their hats in the air at having signed an agreement to collaborate on the building of nuclear power stations. The prospectus is cheerful enough. The companies say that they have between them a good deal of experience in building thermal reactors of several types. The British company has been in gas-cooled reactors from the start. The companies from the mainland, and particularly Interatom and Kraftwerk Union, have a good deal of experience with the construction and operation of water-cooled reactors to an American pattern. But the Nuclear Power Group is also the principal contractor for the prototype fast reactor due to come into operation at Dounreay in Scotland towards the end of next year, while other members of the group are involved with a similar scheme in Germany. Between them, it is clear, the five companies now collaborating will be more effective as a consortium than they could be separately. In Germany, moreover, there is enough work in prospect to keep Kraftwerk Union busy until the late seventies at least, with a mixed programme of pressurized and boiling water reactors. Yet it remains a sad truth, as it has been for some years now, that the nuclear business in Europe has not developed as vigorously as everybody expected only a few years ago. What has gone wrong?

The present condition of the nuclear power industry is a striking illustration of how promise can be dulled by events. In the mid-fifties, to be sure, everybody was delighted with the then government's White Paper which declared its faith in an ambitious programme for the construction of nuclear power stations. In the event, the programme turned out to be too ambitious. Especially because what seemed to be an impending shortage of fossil fuel failed to materialize, the government found itself having to ask the Atomic Energy Authority and the contractors to think again. By the early sixties, however, a second proposal for a programme of nuclear power was cheered on by everybody concerned, and eventually the Central Electricity Generating Board found itself buying reactors of the advanced gas-cooled type which have turned out to be more reliable than the sceptics feared but less decisively advantageous in money terms than some had hoped. But now there seems to be another hiatus in the programme. In part, the Central Electricity Generating Board is restricted by the way in which the demand for electricity has fallen short of earlier estimates. With the present high cost of borrowing money, nuclear power stations with their comparatively large capital cost per megawatt installed, are plainly at a disadvantage compared with conventional generating stations. But there are also technical uncertainties ahead. Should the generating board wait until it can commission fast reactors in the late seventies? The programme now agreed

is that a decision should be made in 1974 (when the prototype fast reactor will have been in operation for a year) about the construction of a full-scale fast reactor power station that will serve to demonstrate what kinds of machinery like this will be needed in commercial service. Or should it take its courage in both hands and build some new type of nuclear reactor, either a high temperature reactor along the lines of the Dragon experiment or a water-cooled reactor on an American pattern, or even possibly a full-scale version of the steam generating heavy water reactor on which the Atomic Energy Authority has in the past few years lavished a good deal of its attention?

The dilemma is real enough, for these are difficult technical choices to make. Nobody expects that the cost of electricity will be much reduced if the advanced gas-cooled reactors now coming into service are succeeded by some other type. There are, however, certain advantages in breaking new ground (although it seems to be accepted that the cost of developing fuel for a commercial version of the Dragon reactor would be a considerable burden). There are also advantages in playing safe and sticking with established reactor types. In short, whatever decision is made about the kind of nuclear power stations to be built in Britain between now and the end of the decade, it will have to be an awkward compromise. But that is no reason for not making a decision. For several months now, a committee of the Department of Trade and Industry has been brooding on the subject. So much time has gone by that outsiders may be forgiven for supposing that any decision, even a decision arrived at by throwing dice, would be better than the indecision which now keeps both the contractors and their only customer in a demoralizing limbo.

The question of what is to be done for the eighties is on the face of things much simpler. Everybody agrees that by then there will be fast reactors in service. The experience of the past few years has shown, in Britain and Germany at least, that the problems of operating liquid metal cooled fast reactors are by no means beyond the range of the technical skills of the nuclear industry. In both countries, considerable sums are now being spent on the research and development needed to bring the reactors to the starting line. Conceivably, there could be hidden obstacles that will bring much current planning to an awkward end but that prospect is not any more real with fast reactors than with other kinds of advanced technical projects. And the chances are high, of course, that consortia such as that now formed on a European basis will be well placed to bring together the fruits of the applied research which has been carried out in several European nations. The trouble is, of course, that nobody at this stage can be sure what future there will be for the products which they may have to sell in the eighties and thereafter. For one thing, there is still no assurance that the supposed free market in nuclear materials which is catered for by the Euratom treaty will ever come to pass in practice. Will, for example, the Central Electricity Generating Board ever find itself placing orders with con-



struction companies in Germany, France or Italy? Will the corresponding utilities on the mainland ever think of using for the same purpose the Nuclear Power Group Limited or the British Nuclear Development Company Limited, its rival in Britain? The answer is that in the last resort, chauvinism will probably prevail. The chances are that if there is a field in which last week's new consortium will win business for itself, it will most probably be outside Europe.

Yet is this not a great misfortune? Is it not high time that a part of the understanding between the countries of the European Economic Community, with the would-be members included, should be that capital equipment installed by governments, public utilities and national airlines should be bought wherever in Europe it can be had most cheaply? This provision would among other things

ensure that the larger market which theoretically exists was actually accessible to industrial companies in Europe. To be sure, such an understanding would have to be negotiated quite separately from the treaties which exist at present, and which do not prevent public authorities from buying local goods, whatever their cost. It is also plain that such an agreement would be hard to reach, for a fondness for national products is deeply ingrained in most governments. Yet it is clear in nuclear power as in many other fields—aircraft and telecommunications are two obvious cases—that if companies cannot enjoy to the full the freedom to sell their skills throughout western Europe, many important economic advantages will be lost. Is this not an issue that should be taken up immediately and quite independently of the discussions under way on the enlargement of the EEC?

Making Policy Explicit

THE latest annual report of the Medical Research Council (see page 213) is a refreshing break with tradition. In many significant ways, the council has broken out of the convention which in the past has allowed it to dwell somewhat smugly on its past successes. To be sure, everybody agrees that the Medical Research Council is one of the most efficient organizations of its kind. It is also a simple truth that to say too much about intentions is to give hostages to fortune. Yet it will help enormously in the better understanding of what the council is like that it should now have given some of its reasons for believing that research should be developed in certain chosen fields. By doing so, of course, it gives up the convention that a research council should back good ideas and bright people, whatever their inclinations in research. Instead, it seeks now to encourage work in fields such as mental health, drug dependence, population control and arterial disease. Nobody doubts that the genteel persuasion which the research councils exercise will be able to bring about shifts in the directions in good people's interests without stifling initiative or the spirit of research.

The device which the Medical Research Council has adopted for charting its course for the future is the setting up of specialist working parties to survey the problems and opportunities in different fields of research. On balance, the working parties appear to have put forward entirely sensible proposals. There are, for example, obvious reasons why the council should make special efforts to support research in arterial disease, for this is bound to be the largest group of intractable causes of death in advanced societies for many years to come. Population control is another field in which medical research can do much to alleviate social problems, but in both cases it is to be hoped that an essential part of the council's planning consists of a close coordination with similar programmes of research elsewhere, especially in the United States. The programme for research in mental health is potentially more difficult to grapple with. Obviously there is room for more and better clinical psychiatry. Obviously the time is coming when the biochemical attributes of nervous function may be relevant to the understanding if not the treatment of mental disease of some kinds, so that much of the research that is sponsored in these fields may be quite fundamental in character. But there remains the

question of what distinctive flavour a research council operating in Britain might be able to provide for its programme of research in mental health. May this not be an opportunity for looking for a closer integration of psychological and psychoanalytical skills with more orthodox medical practice? These are fields in which the council has wisely recognized that the interest of the British scientific community can be harnessed to social need.

It is significant that in the report the council makes a case for continuing its programme of research in tropical medicine, and of course this can easily be justified by the recognition that infectious diseases can leap across national frontiers. But there is a more telling if less parochial reason for hoping that research in tropical medicine will continue to flourish. In the past few years, and with the decline of western European influence in Africa, the old proving ground for tropical medicine, there has been a loss of momentum in an important area of research. It would be entirely proper if the Medical Research Council were to bend whatever rules may at present inhibit so as to make sure that this work continues.

100 Years Ago



Western Chronicle of Science

I WOULD beg to be allowed one or two remarks with reference to the very favourable review of the "Western Chronicle of Science" which appeared in last week's *NATURE*.

It is not a "common Cornish habit to hang heavy jackets, great-coats, &c., on the lever of the safety-valve," and the farmers do not, *as a rule*, "mix guano with lime a few days before applying the manure." The editor has seen both these absurdities performed, and has used them as beacons to warn young men what to avoid. I may also remark that Mr. Williams's Paper is on *Scientific Mining* and not *Scientific Nursing*.

Falmouth, July 22

J. H. COLLINS

From *Nature*, 4, 243, July 27, 1871.

OLD WORLD

ESRO

Decisions in Principle

from a Correspondent

EUROPE took an essential step nearer transferring air traffic control links to space satellites at the end of last week. This is expected to enable ten times as much air traffic to cross the Atlantic as at the present busiest levels, and increase safety. The plan is a joint scheme with the US and decisions on European intentions and financing were taken at a two-day meeting of the Council of ESRO (European Space Research Organization) held at its technology centre in Holland. This follows 18 months of talks and negotiation with the Americans, culminating in a conference in Washington a month ago where decisions in principle were taken for a world wide aeronautical satellite network comprising at least three equally spaced satellites. Other states including Australia and Japan took part.

A "return match" meeting to clinch the project is scheduled for Madrid for early August.

Meantime, ESRO's council made a breakthrough last week in putting European space collaboration on useful satellites on a firm footing, covering other projects besides air traffic control. France, which threatened last winter to withdraw completely from the organization, is now securely back and is one of the European "big four" that will underwrite the new ESRO Space Applications Programme. Britain, Germany and Italy are the others. These countries have agreed together to fund \$70 million per year as a minimum for application satellites from 1974 till 1980. In the interim period, these countries have undertaken to provide between them \$27 million (1972) and \$53 million in 1973 to get the application programme started. The other six member countries are to contribute to whatever applications satellite they fancy "à la carte". The big four will support all of them. As at present envisaged this will be apart from the air traffic control project, the TV and telephone communications satellite scheme for Europe, and a meteorological satellite system.

To include space applications in its activities requires the original ESRO convention to be rewritten; this has been put in hand through a working party due to report by the end of 1971. The applications work is extra to the scientific programme, now committed at about \$30 million a year.

Officials see in last week's agreement and amiable atmosphere a solution to the perennial difficulties of European

space collaboration. It was probably significant that the ESRO council's discussion was able to complete its business without getting involved in any way with the controversial matter of space launchers and particularly the post Apollo programme that has acted as a spanner in the works of the Ministers' European Space Conference for the past 18 months. A lengthy communiqué was issued at the end of the meeting outlining the seven heads of agreement.

MRC

More Revelations

AS well as providing a detailed account of how Britain's largest medical research organization spends its money, the annual report of the Medical Research Council is rapidly becoming a good read in its own right. The report for 1970-71, published last week (HMSO, 85p), describes the overall pattern of research sponsored by the council in its own establishments and elsewhere, but there is also the third in a series of articles through which the MRC seeks to make plain the rationale underlying the organization of its research programme. Last year, the relationship between council policy and the universities came under scrutiny (see *Nature*, 227, 322 and 324; 1971); this year it is the turn of the council's own research units and establishments. By way of bonus there is the text of the MRC evidence to the committee on privacy. The report is about 40 per cent longer than last year's; the price, however, has almost doubled.

The council seems to be expanding its new found policy of letting its customers into the kitchen. In addition to the account of its own establishments, this year's report includes a section which seeks to trace the development of MRC policy with respect to research; in other words, a brief guide to the research topics which the council is currently eager to support. Chief amongst these are mental health, drug dependence, population control and arterial disease. Similarly, the MRC is anxious to encourage and increase the number of active researchers in psychiatry, clinical neurology, obstetrics and gynaecology, dermatology, clinical pharmacology, dentistry, mycology and epidemiology.

In 1970-71, the MRC spent £22,657,537, 92.6 per cent of which came from the Parliamentary grant-in-aid. Plainly, inflation is more than keeping pace with the council's economists, however, for the total income includes a supplementary grant of £1,385,000 to meet the cost of unbudgeted salary increases awarded to

the staff during the year. The table shows how the cost to the government of supporting the MRC has risen since 1966-67. Nevertheless, the council seems to occupy a favoured position in the government's financial scale, for with an increase in support of almost £4 million, equal to 7.5 per cent in real terms, the council is clearly holding its own where other organizations must finger nervously their purse strings.

The council claims that in implementing its research policies it "prefers where possible to work through the universities", but nevertheless the lion's share of its funds remains earmarked for its own establishments. The largest of these, the National Institute for Medical Research, took £2,735,721 in 1970-71 and its clinical counterpart, the Clinical Research Centre, claimed £2,029,310. Between them, the other 76 research units accounted for £9,936,784, while in contrast, research grants for work done in hospitals or in the universities amounted to only £4,831,365, 22.9 per cent of total expenditure. This sum represents an unprecedented increase of 42.3 per cent over the amount awarded in the previous year, a consequence, no doubt, of the surge of grant applications from university scientists unable to obtain support from their more usual sources.

There is no clear sign that the council intends to divert very much more of the funds in its gift towards research in the universities, a policy which is only partly explained by the reasons it has set out to justify supporting research in its own establishments. These are of three kinds, the

COMPUTERS

Hungary Looks Ahead

COMPUTERIZATION and the training of computer specialists feature prominently in the new (fourth) five-year plan in Hungary. The actual operation of the plan itself will involve more than 300 computers, a large proportion of which will be imported from other Comecon countries. During the next five years some 20,000 computer specialists are to be trained. A special educational centre is to be built, equipped with closed-circuit television, and consisting of at least twenty or thirty lecture halls, classrooms, laboratories, mechanical workshops and a central programming point. A ten-storey hostel block to serve the centre will also be built. By using prefabricated units it is hoped to complete the computer training centre by 1973.

Government Aid to MRC for 1966-71

Year	Parliamentary grant: £m	Percentage increase over previous year	
		Actual	Expressed in terms of constant prices
1966-67	11.825	17.2	12.2
1967-68	13.758	16.3	13.0
1968-69	15.230	10.7	6.0
1969-70	17.591	15.5	8.0
1970-71	21.055	19.7	7.5

large interdisciplinary complexes of which there are three in Britain, the NIMR, the CRC and the Laboratory of Molecular Biology in Cambridge; the smaller, specialized units such as the Radiobiology Unit and the Toxicology Unit; and the overseas units. The reasons why the council spends about 75 per cent of its income in these units are two-fold; it is the only way to support important research that could not easily be fitted into a university or medical school; and it is the only practical way to support adequately individuals "of really exceptional ability". The report says that "a council establishment is created only when the purpose in question is unlikely to be attained, or attained so fast or so effectively by any other means".

The council's evidence to the committee on privacy understandably treads a careful path between the value of the analysis of medical records in the study of the aetiology and epidemiology of lung cancer, leukaemia and other similar diseases, and the safeguards necessary to protect the individual against abuses of the system. The first step towards resolving the debate has been taken, however, for the council has appointed a committee to prepare a draft code of practice for the guidance of its own staff working in this field.

SCIENTISTS' PAY

Protest Meeting

THE first public meeting to discuss the British Government's response to the pay claim of members of the scientific civil service was held in the hall of Fitzharrys School, Abingdon, on Friday evening, July 16. The meeting was very well supported and the 400 people present gave an enthusiastic welcome to the assistant secretary of the Institution of Professional Civil Servants (IPCS), Mr Colin Waters, and to the Member of Parliament for Abingdon, Mr Airey Neave, both of whom addressed the meeting. The purpose of the current series of meetings that will culminate on August 10 with a gathering at the Central Hall, Westminster, is to publicize the dispute between the scientists and the government in advance of

the official tribunal that will sit on August 12.

The background of the dispute is that in May the IPCS presented its case to the government for a revision of the pay of the scientific grades; at the end of June the government made a pay offer that included no increase for the higher grades and an increase for the other scientific staff that was well below that demanded by the institution. The reasons for the government's offer are primarily based on the principle of "horizontal comparison" of salaries with similarly placed scientists outside the scientific civil service. Such a comparison has been standard practice and was made after investigations by an independent Pay Research Unit. The latest compiled report shows that the government scientist is as well off as his counterpart in industry. The IPCS counters this by saying that the comparison with industrial salaries is nowadays unfair as a great number of industrial firms base their salary structure on what the government pays its scientists and therefore it is no wonder that the comparison shows equality. The IPCS also states that there are other factors to be considered in the comparisons not least of which are the lack of opportunities for the top government scientist to move into management or other positions.

The presence of so many people at the Abingdon meeting demonstrated the displeasure of the working scientist with the government's offer. Only a solitary voice, however, was raised to complain about the decision of the IPCS not to take industrial action whatever the outcome of the tribunal and the meeting wholeheartedly supported the institution's officials in their campaign to obtain a better deal. The loudest cheer of the evening was for the member of the audience who said that scientists must not be subjected to another pay research exercise and that a fairer method must be devised to determine the basis of the salaries of government scientists in future.

Mr Airey Neave said afterwards that he was encouraged by the strength of the support and the knowledge that the scientific staff in the government's employ were very dissatisfied with the offer made to them gave him increased strength in his negotiations with the government on their behalf.

MINERALS

Mixed Blessings

THE conservationists and protectors of the British national parks received a slap in the face last week with the announcement by the Secretary of State for Wales, Mr Peter Thomas, that Rio Tinto-Zinc Ltd could continue prospecting in the Snowdonia National Park. This announcement came a week after the Minister for Industry, Sir John Eden, had announced a subsidy scheme to encourage mining companies to prove the mineral resources of Britain. Although the two decisions are unconnected, they certainly demonstrate that the national parks, in contrast to the ones in the United States, are not to be regarded by the government as immutable tracts. This is in spite of assurances given by both Mr Thomas and Sir John that their respective decisions do not give any assurances that permission to mine within the national parks will eventually be given.

It is, however, inconceivable that permission to prospect would be granted if an absolute bar on mining within the confines of the national parks were contemplated. Similarly, Sir John Eden's plan to develop mining within Britain would lose all its relevance if the national parks were held to be outside the allowed mining areas. It must be noted that a precedence for mining within a national park has already been set with permission being granted a year ago for two potash mines to be sited within the Yorkshire National Park.

Rio Tinto-Zinc is now given permission to prospect for gold in the Mawddach estuary and for copper at Coed y Brenin near Dolgellau. The prospecting will necessitate drilling boreholes and the ministry inspector has limited the number of drills that can be used in the estuary to two and the number of boreholes to be dug to twelve, whereas no more than four drills can be used simultaneously at Coed y Brenin. At the inquiry, the company stressed that the drills would make less noise than a farm tractor and would create no nuisance and would not pollute the estuary. The inspector, in his report, noted that the drill rigs would not be an eyesore and would pass unnoticed amid the landscape.

With these set conditions the conservationists have been partly mollified but they cannot rest because of the possibility of large scale copper mining in Snowdonia some time in the future. The mining of copper of low quality, as is expected to be found in North Wales, will involve large scale open cast working with wholesale disruption of the terrain. It will of course be almost impossible to heal the scars suffi-

ciently to completely restore the pre-mining conditions. Mining in the Mawddach estuary, however, will probably cause no such wholesale destruction as RTZ's plan is to sift the estuary sands for gold washed down from the mountains by the Mawddach River.

There is no doubt that if substantial gold and copper deposits are found they will be regarded as mixed blessings. Britain's imports of non-ferrous metals cost £600 million annually and any reduction in this bill is to be welcomed. Nobody has a figure for the value of keeping the national parks unspoiled.

EDUCATION

Curriculum Analysis

AFTER five years of quiet but rather troublesome rumination, the Department of Education and Science has finally produced the second volume of results of its *Survey of the Curriculum and Deployment of Teachers 1955-56* (HMSO, £2.50). The first volume, published in 1968, covered the qualifications and deployment of teachers in the various types of secondary schools; the second completes this aspect but concentrates chiefly on the second aim of the survey, documenting detailed statistical information about the structure of the curricula and the sizes of teaching groups.

A total of 448 grammar, modern and comprehensive schools were selected for the sample and the information was obtained from the teachers by questionnaire. The returns yielded two million separately recorded items of information concerning 218,747 pupils. It is hardly surprising, therefore, that a large number of errors came to light as the individual curricula were constructed and it was the eradication of these apparently that took the time. In spite of the delay, the department hopes that the analyses are still generally relevant to the present structure of education.

The survey starts by analysing the science curricula of pupils in all types of schools during their first five years, revealing that some science is included in the curricula of at least 85 per cent of the students in schools, and continues with a breakdown of the percentage of pupils studying specified subjects. It was found, for example, that in the grammar school sixth forms, 15 per cent chose to study biology, botany or zoology at advanced level, 21 per cent chose chemistry, 32 per cent chose mathematics and 28 per cent chose physics. The corresponding figures in the comprehensive schools were biology 8 per cent, chemistry 12 per cent, mathematics 20 per cent and physics 17 per cent.

As far as the balance between science

and arts teaching is concerned, the survey provides a clear picture of the situation as it was five years ago and it is therefore a good starting point for further study of the recent trend towards the arts and social sciences. In 1965, there seems to have been a slight bias towards science subjects in the grammar school sixth forms. Approximately 28 per cent studied science subjects alone, 25 per cent studied arts subjects and 8.5 per cent studied a mixture; of the rest, 1.8 per cent studied social sciences alone, 22 per cent combined social sciences with science subjects and 9 per cent combined social sciences with arts subjects. Comparable statistics are given for the other types of schools, maintained and non-maintained, and for boys and girls separately.

GLASSHOUSE CROPS

Laboratories Opened

from a Correspondent

NEW methods of pest control will be one of the primary concerns in two new laboratories for research in plant physiology and plant pathology at the Glasshouse Crops Research Institute, Littlehampton, which were opened on July 14 by the Secretary of State for Education and Science, Mrs Margaret Thatcher. The laboratories, which together with equipment have cost £250,000, comprise the plant physiology laboratory and the adjoining controlled environment building. They will provide facilities for the study of the growth and development of glasshouse and other protected crops. The scientific approach is multidisciplinary and includes plant physiologists concerned with biological aspects of crop response to the environment, and physicists studying both physical aspects of crop growth and the factors that affect the interaction between crops and the environment. The same building houses biometricians who simulate the various growth processes of crops, using mathematical models to achieve a better understanding of how to exploit the crop environment to give maximum productivity.

In the controlled environment building, six cabinets provide precisely controlled environments for the study of plant responses to light, temperature, humidity and carbon dioxide concentration. There are also three larger airtight cabinets in which groups of plants can be studied, so that the carbon dioxide exchange of the growing crop can be measured. There are naturally lit growth cabinets in which the conditions are less precisely controlled but the environment is more natural. For studies of post-harvest

physiology there are six controlled-temperature stores and two "keeping quality" rooms in which harvested produce, including cut flowers and mushrooms, can be held.

The laboratory complex for plant pathology was designed to extend existing facilities to house all the staff in the entomology, nematology, virology, and mycology and bacteriology departments. The research programme is concerned with improving techniques for the control of glasshouse and mushroom pests and diseases and especially with the development of integrated methods which harmonize chemical and biological control. The use of insect diseases as biotic pesticides is being studied in a new insect pathology unit which occupies separate laboratories designed to facilitate the handling and culture of pathogenic organisms in sterile conditions. The staff of this unit have come from the Agricultural Research Council's Unit of Insect Physiology at Cambridge and from the Pest Infestation Laboratory at Slough, to give the institute the largest centre for research on biological control of agricultural and horticultural pests in Britain. They will aim to exploit arthropod predators and parasites, nematodes, viruses, bacteria and entomogenous fungi as agents of biological control.

Recognizing the advantages of concentrating the work at one centre, the Agricultural Research Council has taken into account concern about unwanted side effects of chemical control. It has approved a programme of research which will provide for the development of biological and integrated control methods for any agricultural or horticultural pest and will not be restricted to pests of glasshouse or mushroom crops.

NUFFIELD FOUNDATION

Sponsoring Innovation

THE Nuffield Foundation has earmarked £95,000 to support a new project which it modestly hopes will yield "some coherent sense of what . . . higher education comprises". The plan is that a group headed by Liam Hudson, professor of educational sciences at Edinburgh University, will, starting this autumn, study a number of university departments to identify the chief problems which crop up in undergraduate teaching. The foundation aims, furthermore, to go some way towards solving these problems, principally by providing funds to enable the staff of the departments to design teaching innovations and put them into practice. The last step in the Nuffield group's programme will be an appraisal of the consequences of these innovations.

NEW WORLD

Agricultural Chemicals Fall Foul of Nader's Raiders

by our Washington Correspondent

A BITING attack on the US Department of Agriculture and on some meat and agricultural chemicals corporations was made last week by Ralph Nader's Center for Study of Responsive Law. Two years' work by a task force of scientists and lawyers working under Nader's auspices has uncovered a wealth of alleged malpractices and shortcomings in the machinery for enforcing standards of meat quality which seem to have made the Wholesome Meat Act of 1967 something of a misnomer. The task force charges, in a report made public last week*, that residues of pesticides, antibiotics, hormones, bacteria and other hidden contaminants may have found their way into meat and meat products because (1) monitoring of such contaminants is totally inadequate; (2) tests sufficiently sensitive to detect potentially harmful levels of such contaminants are not available; (3) pesticides are often misused—frequently with the connivance of the chemicals companies—and the machinery for banning potentially harmful pesticides is cumbersome and heavily biased in favour of the manufacturer; and (4) there is a confusing and damaging division of responsibility between different agencies in the Federal government for setting and enforcing quality control standards. But the chief complaint of the task force is that the combined lobby of the meat and chemicals industries is so powerful that regulatory agencies are firmly in their control.

The chief focus of discontent in this sorry catalogue of woes is the Department of Agriculture, described by Nader at a press conference last week as "ridden with crime". And, according to Harrison Wellford, who directed the study, "the most challenging and risky decision-making outside of national defence is often found in federal regulation of the technology and corporate practices of food production, particularly the application of chemical technology to food and agriculture". But the Department of Agriculture does not see it that way, for it put out a statement last week in reply to the report which suggested that the task force dealt with "certain specific problem areas which are not typical either of the conditions that exist in the depart-

ment or in the food industry". Typical or not, some of the problems discussed in the task force report cut right across the work of the department and are a cause of concern to scientists and, increasingly, to the public.

One such problem is the use of antibiotics in animal feedstuffs. Nearly 80 per cent of the meat, eggs and milk consumed in the United States comes from animals that have been fed for all or part of their lives on medicated feeds, and the stake that the drugs industry now has in this business is such that more antibiotics are sold to farmers than to hospitals. Concern over the use of antibiotics in agriculture centres around the possibility that drug resistant strains of bacteria may develop in animals, and that their resistance may be transferred to human germs. What the task force is particularly concerned about is that the regulatory controls are more than shaky: "while the FDA (Food and Drug Administration) must approve each antibiotic for safety and efficacy, farm use of these drugs is hardly controlled at all". The FDA approves the use of drugs and sets a safe level of tolerance in meat and meat products which the Department of Agriculture analysts then monitor. But what sometimes happens in practice is that drugs are approved by the FDA before the Department of Agriculture has developed techniques to test for their presence in meat. Such is the case with the nitrofurans drugs which are used to control coccidiosis in poultry, and this situation of the right hand not knowing what the left is doing is made more alarming by the fact that the FDA now believes that some nitrofurans may be carcinogenic.

The task force recommends that in view of these occurrences, the FDA should not allow the use of any drug in animal feeds for which the Department of Agriculture has not developed sufficiently sensitive detection techniques, and in the long run, monitoring of food for poisonous contaminants should be centralized in one health-oriented agency. Moreover, like the Swann Report in the UK, the task force suggests that the use of "human antibiotics such as penicillin and tetracyclines as growth promoters and stress relievers for livestock poses an avoidable human hazard which the public should not tolerate, particularly when

there are substitutes available which have no use in human medicine".

The use in the United States of a hormone, diethylstilboestrol (DES), is an even more clear-cut example of commercial profits being weighed against potential human health hazards. DES is mixed with the feed of nearly three-quarters of the cattle slaughtered in the US, and it is used to promote growth. It has been estimated that DES adds \$90 million a year to the profits of cattle growers, and the task force quotes the National Cattlemen's Association as saying that beef prices would increase by up to ten per cent if DES were banned. Yet it is the only chemical that is widely used as an animal drug for which there is strong evidence that it is carcinogenic both in test animals and in man.

McElroy Leaves NSF

by our Washington Correspondent

DR WILLIAM D. McELROY is to leave his post as Director of the National Science Foundation on February 1, 1972, to become Chancellor of the University of California at San Diego. He will leave the NSF after only two and a half years in office; the usual term is six years. Spokesmen for the NSF emphasize, however, that McElroy is not leaving because of any major disagreement with the Administration, and he has in fact decided to stay until the Administration budget for 1973 has been completed.

Since taking office in 1969, McElroy has presided over an expanding budget which has risen from \$432 million in 1969 to about \$600 million for 1972; the 1972 budget alone represented an increase of \$100 million over 1971, chiefly to salvage some projects abandoned by other agencies. His appointment to the chancellorship of San Diego was unanimously agreed at a meeting last week. McElroy is a biologist, and headed the biology department at Johns Hopkins University before he was appointed by Nixon to the NSF in 1969.

**Sowing the Wind*, by Harrison Wellford. Center for Study of Responsive Law.

A study conducted by the National Cancer Institute in 1956 found that a dose of 0.07 micrograms of DES caused uterine tumours in 50 per cent of mice treated—which makes it a particularly powerful carcinogen—but the sampling techniques used by the Department of Agriculture can only detect DES at the level of 0.9 micrograms per pound of meat—fourteen times the dose used in the NCI study. Describing the testing procedures for DES as “absolutely hopeless”, Dr Samuel Epstein, of the Laboratories of Environmental Toxicology and Carcinogenesis at the Children’s Cancer Research Foundation in Boston, suggested last week that “there is no question that it is carcinogenic in animals and it is highly likely to have caused some human cancers”. The clinical evidence for the carcinogenicity of DES in humans is based on the diagnosis at Massachusetts General Hospital in April this year of an extremely rare cancer of the vagina in seven young women. The common factor in these cases was that, nearly 20 years previously, their mothers had been treated with stilboestrol to prevent miscarriage. Since that discovery, Dr Epstein said that reports of this rare cancer are “just pouring in”.

The Department of Agriculture is clearly in a cleft stick over the use of DES, for on the one hand it is faced with an extremely powerful lobby from farmers and drugs manufacturers to continue with its use, but on the other, testing techniques are simply incapable of detecting potentially dangerous contamination. The task force therefore recommends the adoption of a crash programme for developing more sensitive testing techniques, and if DES residues do appear regularly in meat after a more sensitive method of analysis is introduced, then the drug should be banned. In making this recommendation, however, the task force does not even go as far as the governments of twenty-one other nations, which have banned outright the use of DES, and Sweden and West Germany have even banned imports of American beef because of the possibility that it may contain residues of the hormone.

As for pesticides, the task force predictably castigates the chemical manufacturers for a variety of malpractices ranging from irresponsible marketing to using delaying tactics in the procedure for banning potentially dangerous chemicals. But the Department of Agriculture also comes in for criticism for foot dragging in its promotion of alternatives to pesticides and for its handling of the campaign to eradicate the fire ant from some southern states. At the root of the problem, however, lies the weakness of federal regulation of pesticide hazards, for without sufficiently strong enforcement laws, the

chemicals companies cannot be expected to limit their profits from the lucrative pesticide market unless forced to do so by a powerful regulations machinery.

The Pesticide Regulation Division, which was transferred last year from the Department of Agriculture to the Environmental Protection Agency, is responsible for these regulations. “The decisions of this little agency,” the task force points out, “with a professional staff of less than 200, affect the health and environment of American citizens on a scale comparable to that of the Food and Drug Administration, the National Air Pollution Control Administration and the Federal Water Quality Administration”. The chief difficulty under which the agency is labouring is that pesticide regulation must justify sacrificing a concrete short-term loss in profits for a vague, long-term public health hazard which is, in any case, difficult to detect. But the main problem lies in the regulatory machinery itself, which the task force points out is heavily weighted in favour of the manufacturer. The Federal Insecticide, Fungicide and Rodenticide Act, for example, leaves the Pesticide Regulation Division free to exercise its discretion over a broad area of regulation and the agency frequently accepts a chemical as fit for use solely on submissions from the manufacturer. “The Pesticide Regulation Division has been criticized for accepting at face value testing data which are shallow and incomplete. PRD rarely conducts independent tests of its own, and does not regularly solicit comments from outside scientists,” the task force reports. Moreover, if the use of a chemical is cancelled by the Environmental Protection Agency, the manufacturer has the right, under the Insecticide, Fungicide and Rodenticide Act, to petition for enquiry by a scientific advisory committee, and until action is taken on that committee’s report, sale of the chemical can continue. Two bills introduced into the Senate, in the names of Senator Gaylord Nelson and of the Administration, seek to remove this right, however, and Nelson’s bill also asks for tests to be conducted by the PRD on all chemicals submitted for approval. The task force report may conceivably have some effect on the passage of these bills.

Turning to the campaign to eradicate the fire ant from some southern states by use of Mirex pesticide, the task force heaps criticism on the programme and on the Congressional lobby that has ensured its continuation. The programme seeks to remove the ant from southern states, where it was introduced from South America some fifty years ago. Since 1957, the Department of Agriculture has launched campaigns to get rid of the fire ant and, in spite of

a report published in 1967 by the National Academy of Sciences which stated that eradication is not biologically or technically feasible, the department, at the instigation of the House Appropriations Subcommittee on Agriculture, has continued to spread Mirex over the states. The task force charges that the campaign has been turned into a political battle by Phil Campbell, Undersecretary for Agriculture, and by Jamie Whitten, chairman of the House Appropriations Subcommittee on Agriculture. A court action seeking to block the programme brought against the Department of Agriculture by the Environmental Defense Fund failed in April this year, and the agriculture appropriations for 1972 contain a \$7.8 million funding for the project. Last week, however, funding for the programme was challenged in the Senate through an amendment proposed by Senator Nelson seeking to delete the funding for the programme from the agriculture appropriations, but it was defeated by 53 votes to 28.

According to the task force, the majority of abuses perpetrated on the housewife through sale of contaminated meat, and on the environment through overuse of pesticides, arise because the interests of the consumer are no match for the powerful agribusiness lobby on Capitol Hill. The suggested remedy is to set up an independent consumer protection office to channel the interests of the consumer through the courts, Congress and federal and state agencies. “The substantive rights of consumer laws are hoaxes when procedures of delay, preferential access and secrecy keep the public from exercising them,” the task force acidly states.

NASA

FAS Opposes Shuttle

by our Washington Correspondent

THE Federation of American Scientists, a small but intellectually powerful group of scientists, has voiced its opposition to NASA’s plans for constructing a re-usable space shuttle. In a statement issued last week, the federation states that the shuttle will not do anything that is different in kind from what can be done already, and that the economic arguments used to justify the project do not stand up to close scrutiny. The FAS has therefore joined the mounting chorus of opposition to the shuttle from some sectors of the scientific community and from a minority of Congressmen who see the project as an unjustifiable waste of public money which can be put to better uses, notably unmanned weather, earth monitoring and communications satellites. The controversy

surrounding the shuttle project is, however, made more difficult by the fact that NASA has staked its future on the project at the expense of cuts elsewhere, for example, in the Nerva nuclear rocket development and in its failure to provide back up vehicles for expensive projects such as the orbiting astronomical telescope and the Mariner missions.

The chief argument put forward by the FAS against the shuttle is that it will not do sufficient business to achieve the economic savings that its supporters claim. Each launch by the shuttle is expected to cost between eight and ten million dollars above the research and development costs, compared with about \$20 million for a launch with the Titan system. Since development is estimated to cost in the region of \$9–10 thousand million between now and 1978, and a further \$4–6 thousand million between 1978 and 1990, only a high level of use will result in cost savings. Dale Myers, Associate Administrator for Manned Space Flight, testifying before the Senate Committee on Aeronautical and Space Sciences, estimated a ten per cent return on investment at a launch rate of about forty a year—sufficient to put a thousand tons into orbit. Pointing out that in 1969 and 1970 the United States launched less than fifty tons of unmanned equipment and 200 tons of manned vehicles, the FAS study emphasizes that by 1990 the unmanned launch rate would be less than 250 tons a year—short by a factor of five of the minimum economical capacity of the shuttle. "Such a programme with presently developed rockets would certainly cost more than shuttle operation," the FAS study concedes, but "that bookkeeping saving is swamped by a heavy development cost to be regained, more than \$0.7 thousand million per year, spread over the period 1978–1990".

The FAS study therefore concludes that the shuttle is justifiable only by a plan for substantial manned space flights in a programme several times larger than the Apollo programme in terms of tons orbited per year. If that is indeed the case, "no one has given any convincing rationale for what man can do in space, for peaceful or for military purposes, which cannot be done more cheaply and with less human risk by instruments, at least near Earth and within the next 20 years," the FAS retorts.

If the FAS had opened hostilities against the shuttle a few weeks earlier, its report might conceivably have had more impact on the appropriations for the project next year, but now NASA's budget has already gone through the House and the Senate. The Administration originally set aside \$100 million for development of the shuttle in 1972, but the authorization agreed by the

Senate early in July gave the shuttle project \$137 million in spite of an attempt by Senator Walter F. Mondale to delete all appropriations for the programme. In the event, the Senate agreed to the shuttle project by a 64–22 vote. In future years, however, budget appropriations for the shuttle will be a more tempting target for, as the FAS points out, this year's spending "is the thin edge of the wedge which will widen eighty-fold by 1978".

One of the factors which may have led to the sanction for the shuttle in Congress is the prospect of more unemployment in the aerospace industry if it were scrapped. Asked whether this factor was considered by the FAS, Professor Philip Morrison from MIT, who chaired the committee which produced the report, said that such considerations were not within his brief, and that, in any case, unemployment in the aerospace industry is to be the subject of a separate study by the FAS. He added that it does not seem sensible to justify such a project solely on the grounds that it would create unemployment if it were scrapped. One part of the post-Apollo project, the space tug, planned to drag payloads from low to high orbits, could bear closer scrutiny, Morrison suggested. So far, the tug has been considered only in relation to the shuttle, but he suggested that it should be considered on its own merits, for it seems to have advantages for commercial use.

HERBICIDES

2,4,5-T Report Attacked

from our Washington Correspondent

THE report of a scientific advisory panel on 2,4,5-T which recommended in May this year that the herbicide should be restored to full use pending further analysis of the health hazards has come under fire from several quarters. The report, which is not meant to be public knowledge until William D. Ruckelshaus, administrator of the Environmental Protection Agency, has pronounced on its recommendations, was attacked last week at a press conference organized by the Committee for Environmental Information (CEI). One of the chief grumbles at the report is that because it has been kept secret, its conclusions cannot be challenged easily by the scientific community, which is relying at present on bootlegged copies circulating illicitly.

The Committee for Environmental Information criticizes the report on five main counts. First, the advisory committee accepts that there is a "no-effect dosage" at which 2,4,5-T has no effect on test animals. But the CEI points out that to obtain a statistically significant result in experiments de-

signed to screen for effects that may be experienced by only a very small percentage of the population would require tests on a sample and control group of several thousand animals. And even then, it would be difficult to isolate any effects caused by 2,4,5-T from background "noise" from other teratogens. The CEI therefore concludes that there is no evidence "which would indicate that low levels of 2,4,5-T are innocuous to animals". Second, the statement points out that there is little evidence that 2,4,5-T breaks down in the environment. Third, dioxin contaminants in the herbicide may accumulate in the soil, and there are no analytical techniques sensitive enough to detect toxic amounts of dioxin. Fourth, the advisory committee implied that since there is no concrete proof that the use of 2,4,5-T in Vietnam is correlated with increased incidence of human birth defects, that no correlation exists. And fifth, the Committee for Environmental Information takes the advisory committee to task for not attempting to consider the benefits of using 2,4,5-T in regard to the hazards associated with its use.

The CEI statement was also supported by Barry Commoner (Chairman, Scientists' Institute for Public Information), John T. Edsall (Harvard), Samuel Epstein (Children's Cancer Research Foundation), Arthur Galston (Yale), Michael Prival (Union of Concerned Scientists), Jeremy Stone (Federation of American Scientists), and Harrison Wellford (Center for the Study of Responsive Law).

Short Note

by our Washington Correspondent

Medical Training

Two bills designed to increase the funding for medical training were passed last week by the Senate. One asks for expenditure of \$5.9 thousand million over the next five years for the training of doctors, and the other seeks to provide an extra \$1.1 thousand million for training of nurses. Both were approved unanimously, and were sent to a conference committee between the House and the Senate, where they will be considered together with similar bills which went through the House the previous week. There is little difference in philosophy between the House and Senate versions, but there is considerable difference in the amount of money requested. The House bill asks for \$2.8 thousand million to be spent over the next three years, and only \$710 million for the training of nurses. It seems that neither the Senate version of the bill nor the House version will be opposed by the Administration.

NEWS AND VIEWS

A Wise Cell which Knows its Parent

A CELL surface must carry its own identity and also a means of identifying other cells. The cell surface must naturally be controlled by specific gene products and diversity among cell surfaces is achieved either by a single gene with multiple alleles or by multiple genes with selective gene activation. Either way the phenotype of cell surface antigens can vary extensively.

Cellular recognition in vertebrates has achieved great sophistication, reaching an advanced stage in the mammalian immune system. Clearly, however, the capacity to elaborate and recognize specific surface structure originated in very simple cells before the evolution of multicellular organisms. Indeed it has been speculated that surface differentiation may have been an essential acquisition for the selective grouping of cells in a multicellular form.

In an article on page 230 of this issue of *Nature*, F. M. Burnet seeks to draw attention to the simple mode of self-recognition exhibited by cells of colonial marine invertebrates and to point to the possible significance of the phenomenon for the evolution of the immune response. Burnet has taken as his principal text a lucid review by Hidemitsu Oka (*Profiles of Japanese Science and Scientists*, 196; 1970) of detailed studies on the genetics of colony specificity in compound ascidians, in particular the Japanese ascidian *Botryllus primigenus*. These animals have several features which commend them for genetic study: they stand high in the evolutionary scale of invertebrates and the larval form has a dorsal nerve cord and notochord. The larvae are active free swimming animals which undergo metamorphosis to sessile adults; these attach to solid surfaces (in nature seaweed or rocks, but for investigation glass plates are used). Each adult then reproduces asexually to give rise to a colony of many interdependent individuals. In this clone of animals all individuals are genetically identical and each is capable of giving rise to a replica colony. The chief disadvantage of working with ascidians seems to be the lack of long term laboratory culture conditions and it is therefore necessary to rear colonies on glass plates in the sea. Bancroft (*Proc. Calif. Acad. Sci.*, 3, 137; 1903) originally observed the recognition process among ascidians. Two fragments of any single colony brought into contact readily fuse together but if the two colonies are of different origin they not only fail to fuse but positively reject each other. This rejection reaction is very clearly illustrated in Oka's review.

Oka started from Bancroft's observation that F_1 colonies (the progeny of a sexual cross between two hermaphroditic colonies) fall into two categories, either fusible or non-fusible with parental colonies. From this type of experiment using fusibility as an assay for colony specificity, Oka showed that the phenomenon is controlled by a single gene locus with multiple alleles. The rules of the game are then simple. Fusion occurs between colonies having one allele in common and rejection occurs between colonies having two different pairs of alleles. The rules for sex are essentially reversed as spermatozoa (carrying only one allele) cannot fertilize ova produced by a colony carrying that allele. This naturally

prevents self-fertilization and promotes the variation necessary for evolution.

Burnet has suggested that this self-recognition and rejection in ascidians represents the type of innovation which may have initiated the evolution of vertebrate immunity. This is an enormous jump and he has merely hinted at possible routes. There is a plea for more data from such simple systems.

The vertebrate immune system is capable of extremely subtle "recognition of foreignness". There appear to be two systems, cellular and humoral immunity—the T and B lymphocyte mediated responses to which Burnet refers (see Roitt, Greaves, Torrigiani, Brostoff and Playfair, *Lancet*, ii, 367; 1969). The two systems are linked by the role of T cells in initiating most B cell responses; the latter culminate in production of circulating antibody. T cells, which mediate graft rejection and graft versus host reactions, do not secrete antibody but function by cell-cell interaction. T lymphocytes probably synthesize surface receptor antibody although this is still a hypothesis requiring final experimental verification. Clearly the invertebrate recognition-rejection mechanism is more analogous to T cell responses. There is, however, a crucial difference. The primitive system exhibits both recognition and rejection only within the genus; when colonies of *Botryllus* and *Botrylloides* come into contact they are oblivious to each other, neither fusing nor rejecting. Thus the recognition system only deals with the "expected". As Cohn has pointed out (*Nucleic Acids in Immunology*, 1968) the immune system deals with the "unexpected". It is clear that despite this ability of T cells to respond to the "unexpected", response to "expected" antigens (that is, histocompatibility antigens of the same species) is spectacular by comparison (Simonsen, *Cold Spring Harbor Symp. Quant. Biol.*, 32, 517; 1967; Wilson and Nowell, *J. Exp. Med.*, 131, 391; 1970). Alongside these observations is the phenomenon exhibited in inbred strains of laboratory animals (McDevitt and Benacerraf, *Advanc. Immunol.*, 11, 31; 1969); the immune responses to various determinants depend on the presence of a dominant autosomal gene mapping in the major histocompatibility locus. With this information in mind, Jerne (*Europ. J. Immunol.*, 1, 1; 1971) has proposed a model for somatic generation of antibody diversity starting from a germ line set of antibody structural genes coding for antibodies against the set of histocompatibility antigens of the species.

Burnet links primitive recognition sites to an idea of histocompatibility antigens and germ line genes coding for antibodies to histocompatibility antigens. Unfortunately there is no evidence to show that the immune response gene is a structural gene for the antibody combining site. It is more likely that the immune response gene exercises control at the level of cell-cell interaction.

It is the positive rejection of non-self, by the ascidians, albeit on a limited scale, which suggests an origin of immunity. The immunocyte with its specialized function and sophisticated mechanism for "recognition of foreignness" could well have had its origins in a primitive recognition system of the colonial ascidian type. The recogni-

tion system evident in the control of vertebrate ontogeny, however, is also reminiscent of the colonial ascidians and of the sponges (see, for example, the recent studies of John, Campo, Mackenzie and Kemp, *Nature New Biology*, **230**, 126; 1971). The experiments of Moscona (*Soc. Exp. Biol. Med. Proc.*, **92**, 410; 1956; and *Proc. US Nat. Acad. Sci.*, **43**, 184; 1957) dramatically illustrate the like-like recognition processes among vertebrate cells. Cell suspensions of embryonic cartilage or kidney tissue exhibit specific cell re-aggregation into aggregates recognizable in terms of their tissue of origin. In mixtures of cartilage and kidney cells specific aggregation of like with like persists. This phenomenon extends beyond species barriers. In mixed cultures of chicken and mouse cells there was preferential association of kidney cells with kidney cells and liver with liver irrespective of the species of origin. The role of like-like recognition is clearly of major importance in a host of systems and the simple ascidian model demands study at the molecular level.

Boyse, Old and their colleagues are involved in mapping cell surfaces both topographically by immuno-electron microscopy and genetically. Surface antigens are present in a patchwork array of discrete areas varying in size according to the antigen and the cell type (*J. Exp. Med.*, **130**, 974; 1969). The extensive phenotypic variation of mammalian cell surfaces is apparently the result of multiple alleles at a limited number of genetic loci coupled with differential gene activity.

In the near future it should be possible to discuss cellular recognition in much simpler terms.

INTERSTELLAR MOLECULES

OH in Radio Galaxies

from a Correspondent

THE search for molecules in space takes on a completely new dimension with the discovery of OH molecular absorption lines in two external galaxies, M82 and NGC 253, by L. Weliachew of the Owens Valley Radio Observatory (*Astrophys. J. Lett.*, **167**, 47; 1971). Following several unsuccessful searches dating from 1967, this is the first observation of interstellar molecular lines originating from outside the Galaxy.

One of the major growth points in radio astronomy at present is the study of molecules in galactic gas and dust clouds. Two years of vigorous activity by astronomers in the United States have led to the identification of twenty distinct molecules in the interstellar medium. In addition to this, several isotopic species, such as the ^{12}C and ^{13}C variants of formaldehyde, have been

detected. The subject grows ever more complex with rival groups now chasing bigger fish. A recent *IAU Circular* (2330) announced the presence of a seven-atom structure—methylacetylene $\text{CH}_3\text{C}_2\text{H}$ —in the galactic centre, as well as hydrogen isocyanide HNC and isocyanic acid HNCO in some bright emission nebulae.

So far, fifteen organic molecules have turned up, but these are all feeble sources and many hours of observation are necessary to record the emission. The strongest molecular emitters are the enigmatic hydroxyl and water vapour sources. These are characterized by enormous intensity, very small angular size, and dramatic variability, and are certainly some kind of celestial maser (see *Nature Physical Science*, **232**, 52; 1971).

There is unfortunately a snag when it comes to searching for emission lines from molecules in other galaxies. Even the most powerful OH emitters in our Galaxy would, if placed in the nearby Andromeda nebula, be way below the

limit that any present radio telescope could detect. A better way of finding OH in distant galaxies is to search for the characteristic absorption features at 1,665 and 1,667 MHz impressed on the continuum radiation from a powerful background source. Historically this parallels the successful method which, in 1963, pinpointed OH in our Galaxy as an absorption feature against Cassiopeia A.

At Owens Valley, Weliachew selected M82 and NGC 253 as suitable candidates for an experiment exploiting the absorption technique: these are galaxies which have moderately powerful radio sources at their centres to act as background radiators. For M82 the OH 1,665/1,667 MHz lines show up in the spectrum at a radial velocity of 240 km s^{-1} and for NGC 253 at 190 km s^{-1} . Taken together with phase angle information these values are strong evidence that the absorption is indeed associated with remote objects rather than our immediate neighbourhood.

It is all too easy to gain the impres-

Selective Antitumour Virus Drugs

To find a drug, as Levinson, Woodson and Jackson have done, which not only selectively inactivates RNA tumour viruses but also has already been clinically tested and used to treat human infections is a rare stroke of good luck. The compounds isatin β -thiosemicarbazone (IBT) and two derivatives, N-methyl IBT and N-ethyl IBT, have been available for several years now; they have a wide spectrum of anti-viral activity directed against both RNA and DNA viruses and N-methyl IBT has been used to treat smallpox. But curiously enough no one had tested IBT and its derivatives for effects on the RNA tumour viruses until Levinson and his colleagues took on the job; they could scarcely have hoped for more promising results than those they report in next Wednesday's *Nature New Biology*.

Their first experiments with N-ethyl IBT suggested that this substance blocked the production of Rous sarcoma virus by transformed cells just as it blocks the growth of viruses of eight other groups. But further analysis showed that cells transformed by Rous sarcoma virus continued to produce virus particles in the presence of the drugs but the particles were rendered non-infectious. Pursuing this paradoxical clue they tested to see if N-ethyl IBT reacted directly with Rous sarcoma virus particles so as to inactivate them and sure enough that was precisely what was happening.

Further tests proved that both N-ethyl and N-methyl IBTs, but not the parent IBT molecule itself, inactivate a variety

of strains of Rous sarcoma virus, avian leukosis virus and mouse sarcoma virus. For example a fifteen minute exposure at 37° C to 40 μM N-ethyl IBT caused more than ninety per cent reduction in the infectivity of Rous sarcoma virus.

By contrast these N-substituted IBTs do not react directly with and inactivate vaccinia virus, poliovirus or Newcastle disease virus. Herpes simplex virus is, however, inactivated when N-ethyl IBT is suspended in phosphate buffer but not when it is suspended in tissue culture medium. Certain herpes viruses have, of course, been linked with malignant tumours.

How do these compounds selectively inactivate the RNA tumour viruses? Levinson and his colleagues have examined two obvious possibilities but have drawn blanks. N-ethyl and N-methyl IBTs do not disrupt Rous sarcoma virus particles neither do they inhibit the reverse transcriptase activity in these viruses. Clearly more detailed investigations are needed to reveal the target molecule or structure in RNA tumour viruses and herpes viruses which renders them susceptible to these drugs.

It is still early days and it would be foolish to raise hopes too high, but these experiments offer a remarkably promising lead. It is now important to establish whether or not N-methyl or N-ethyl IBTs can cause the regression or prevent the growth of, for example, sarcoma induced in mice by mouse sarcoma virus. If such experiments are successful the case for clinical trials with selected human cancer patients may be irresistible.

sion that the molecule hunters are gathering data with no attempt at interpretation. This is emphatically not the case, and a wealth of information is concealed in the line measurements. In this particular case Weliachew has estimated the abundance of OH relative to neutral hydrogen, and for M82 and NGC 253 this is comparable with representative regions in the Galaxy. There is, however, a note of caution: both galaxies have strong radio sources in their centres and so the excitation conditions may not be similar to the Galaxy. Nevertheless the evidence does not point to any completely new type of excitation mechanism for the OH in these energetic galaxies.

VIROLOGY

Links and Rings

from our Cell Biology Correspondent

WHEN small covalently closed, circular DNA molecules replicate, there seems to be an inherent tendency to generate a small proportion of oligomeric complex forms, either continuous ring-shaped DNA molecules two or more times the size of the monomer, or catenated forms, consisting of two or more monomeric rings interlocked like the links of a chain. Such oligomeric forms of the genomes of the bacteriophages ϕ X174, P22 and M13 have been described, as have oligomers of circular mitochondrial DNA from a variety of mammalian cells and kinetoplast DNA from the protozoan *Trypanosoma cruzi*. It comes as no surprise, therefore, that Cuzin and his colleagues have detected complex forms of polyoma virus DNA and Rush, Eason and Vinograd (*Biochim. Biophys. Acta*, **228**, 585; 1971) and Jaenisch and Levine (*Virology*, **44**, 408; 1971) now report both ring-shaped and interlinked oligomeric forms of simian virus 40 DNA among the molecules which can be extracted from African green monkey cells supporting the replication of this virus.

Rush *et al.* find that rings and interlinked oligomers each constitute about 1 per cent of the total DNA extractable from cells supporting SV40 replication. Most of these molecules are dimers but there may also be trimers mixed with mitochondrial DNA which happens to have an identical size. Rush and his colleagues, following the lead of Riou and Delain, who reported that low concentrations of ethidium bromide markedly alter the frequency of oligomeric forms of kinetoplast DNA, added $10 \mu\text{g ml}^{-1}$ of this drug to their cultures of SV40 infected cells but there was no significant effect; oligomers were found at the same frequency as

in untreated cultures. As Eason and Vinograd have reported elsewhere (*J. Virol.*, **7**, 1; 1971), however, ethidium bromide does affect the superhelical coiling of intracellular SV40 DNA; it causes the progeny viral molecules to have a homogeneous superhelix density, the same as that of DNA extracted from SV40 particles, instead of being heterogeneous in this respect.

Similarly to Rush and his colleagues, Jaenisch and Levine report the detection of about 1–2 per cent of circular and interlinked oligomeric SV40 DNAs in infected cells (their electron micrographs of the interlinked forms are particularly elegant and convincing) and they claim some of the ring forms are up to six times the size of monomers. Infectivity tests reveal that each different class of oligomer is infectious and the specific infectivity of these complex forms is more or less that expected; dimeric DNA, for example, has a specific infectivity half that of monomeric DNA.

Cuzin *et al.* detected about 1–2 per cent oligomeric polyoma DNA in mouse 3T3 cells supporting the replication of this virus, but they also found oligomers at a much higher frequency

in 3T3 cells that had been transformed by the polyoma virus mutant *tsa* and then induced to allow replication of the mutant. This observation led Cuzin and his colleagues to speculate that oligomeric polyoma DNAs might be produced by the excision of multiple copies of the polyoma genome, which had integrated in tandem in one or more of the host cell chromosomes. It is of course impossible that oligomeric SV40 DNAs are also produced in this way although there is as yet no good reason to believe that the SV40 genome is ever integrated into its host's genome during replication. The high proportion of oligomers in induced *tsa* transformants may therefore be a unique situation and currently it seems more likely that SV40 oligomers arise either by some aberrant event during replication of the free viral genome or perhaps as a result of some recombination event. Certainly Jaenisch and Levine are thinking along these latter lines, for they are apparently infecting cells with pairs of SV40 mutants in an attempt to decide whether dimeric molecules are produced at replication or by recombination. No doubt we shall not have long to wait to know their answer.

Towards the Genetics of Hormone Action

THE ability of the molecular biologist studying bacterial control mechanisms to isolate and recombine regulatory mutants is a constant source of envy for his counterparts working with animal cells, for whom such techniques are not readily available. As some consolation, however, it is being recognized that a number of inherited diseases in man and other animals may involve regulatory functions. β -Thalassemia has already been successfully exploited from this point of view (see, for example, Nienhaus *et al.*, *Nature New Biology*, **231**, 205; 1971). In a forthcoming article in *Nature New Biology*, Ulrich Gehring and Gordon Tomkins turn with characteristic perspicacity to the inherited defect known as "testicular feminization" for insight into the mechanism of action of steroid hormones. Individuals with this abnormality have an external female phenotype, but an XY genotype, and are insensitive to androgenic hormones, including 5- α -dihydrotestosterone, the presumed active metabolite of testosterone. The defect has also been described in mice, where it is known to be X-linked.

Susumo Ohno, with whom Gehring and Tomkins collaborated, had previously shown that normal mouse kidney is a target tissue for sex hormones, and responds to testosterone injection with

the induction of a number of specific enzymes, including alcohol dehydrogenase. It is worth noting here that this kidney enzyme is coded for by the same autosomal gene as in liver, where it is not under steroid control (Ohno *et al.*, *Biochem. Genet.*, **4**, 565; 1970). In all steroid-sensitive tissues so far studied it had been assumed that the first step in the action of the hormone was its combination with specific cytoplasmic receptor proteins, followed by their association with the nucleus. Gehring *et al.* show that such complexes are indeed formed with dihydrotestosterone in the kidneys of normal X^+Y and X^+X^+ males and females but are present in very reduced amounts in the cytoplasm and nuclei of $X^{Tfem}Y$ mice. It is not yet known if the X^{Tfem} mutation involves a structural alteration, or loss, of one or a class of cytoplasmic dihydrotestosterone-binding protein(s), but what these results do establish is that the combination of the steroid with such molecules is a prerequisite for its correct biological action. The number of these cytoplasmic receptors, their uptake and specific binding to chromatin, and the relation between these steps and transcriptional and translational control are obviously problems that will, in the absence of further regulatory mutants, continue to tax everyone's ingenuity for a long time.

ENVIRONMENT

Mercury Levels

from our Photosynthesis Correspondent

THE build-up of alkyl mercurials in the environment and their toxicological properties was the subject of many of the contributions presented at this year's international conference on environmental toxicity held at the University of Rochester, New York, from June 17 to 19. Dr L. Friberg (Karolinska Institute, Stockholm) and Dr L. T. Kurland (Mayo Clinic, Rochester, Minnesota) emphasized the difference between methyl and ethyl mercury and inorganic mercury as toxicological agents. Only inhaled elemental mercury vapour is comparable with the above organo-mercurials in inducing intoxication, reflecting the ease with which the vapour can diffuse across the alveolar membranes. The absorbed mercury vapour produces its toxic effect after being oxidized to mercuric ions in the body. Dr F. N. Kudsk (University of Aarhus, Denmark) reported that this oxidation occurs chiefly in the erythrocytes—possibly through a peroxidase reaction. He has also used menadione sodium bisulphite to stimulate mercury vapour uptake into the blood and reported that this process is inhibited by 0.4 per cent ethanol.

There have been many clinical cases of elemental mercury poisoning, but they have usually been associated with occupational exposures. Far more serious, however, are the increasing levels of methyl mercury to which the general public are being exposed. Two serious epidemics of methyl mercury poisoning have already occurred in Japan, at Minamata and Niigata, where out of a total of 230 intoxications there were about fifty deaths. Apparently, as Dr A. Jernelöv (Institute for Water and Air Pollution, Stockholm) explained, the reason for the increased levels of methyl mercury in nature seems to be microbial activity in the bottom sediments of mercury polluted lakes, rivers and coastal waters. He reported that the methylation process, at least for *Neurospora crassa*, is associated with the biosynthetic pathways which are responsible for methionine production. The methylated mercury finally finds its way into the food chain through contaminated fish, and several speakers pointed out that methyl mercury is readily absorbed into the blood stream from the intestinal tract. Presumably because of its stability and high lipid solubility the methyl mercury readily diffuses in the body and accumulates to significant levels in the organs and brain. High levels in the brain would account for the neurological symptoms that are associated with methyl mercury poisoning. The biological half-life for the total body

burden of methyl mercury is about seventy days but the half-life for some specific parts of the body, for example, the brain, may be very much longer. Of all the organo-mercurials that are commonly encountered none is more toxic than ethyl and methyl mercury, and as Dr L. J. Goldwater (Duke University, North Carolina) reported, aryl and alkoxyalkyl mercurials have never been reported to produce serious clinical disorders similar to those observed with short carbon chained alkyl mercury compounds.

The clinical symptoms of methyl mercury poisoning were clearly shown in a film presented by Dr M. Berlin (University of Lund, Sweden). He has conducted closely controlled rapid and slow methyl mercury exposure experiments on squirrel monkeys. The methyl mercury was fed to animals in their food to obtain a blood level of about 1,400 ng Hg g⁻¹ blood. The rapidly exposed monkeys became totally blind within three weeks while the slower fed animals suffered from impaired peripheral vision and lack of coordination after eighty to a hundred days. Because of their fumbling they were unable to carry out simple tests although they maintained the motivation to do so. There was, however, no effect on their hearing. Pathological studies on these monkeys by Dr C. A. Grant (Karolinska Institute, Stockholm) indicated that those that had been rapidly exposed to methyl mercury had damage of the central cortex while the longer lived monkeys suffered even more severe brain damage particularly in the sub-cortical region, having degeneration of the peripheral nerves and

dorsal roots. It has been suggested in the past that the toxicological properties of natural and laboratory methyl mercury are different. Dr Grant, however, clearly showed that this is not the case from clinical and pathological studies on rats fed with both forms of methylated mercury.

The way in which organo-mercurials and inorganic mercury act at the cellular and subcellular levels is far from clear. Papers by several renal physiologists indicated that mercurials can act on the transport mechanisms which control the movement of both non-charged and charged solutes across the kidney membranes. For example, Dr J. Vostal (University of Rochester, New York) considered possible mechanisms which may account for the diuretic properties of some organo-mercurials such as chloromerodrin and mercaptomerin. His experiments did not support either the possibility that inorganic mercury released from these agents or that a lock and key mechanism can explain their diuretic action. Papers by Dr A. Rothstein (University of Rochester, New York), and Dr J. Barber (Imperial College, London), reported experiments that showed that both inorganic and organic mercurials can induce permeability changes in cell membranes and interfere with cation transport mechanisms by reacting with surface sulphhydryl groups.

The take home message from this meeting was clear. The careless discharge of mercury into the environment over the past fifty years or so has created serious ecological and toxicological problems that have yet to be solved.

Creep of Ice at Low Stresses

LABORATORY studies of creep are often hindered by the length of time required for the test. This is particularly true at low stresses where a transient creep term dominates and the presence of a small steady-state creep may be masked.

The creep of polycrystalline ice has been studied in the laboratory, and the results have been applied, sometimes in extrapolated form, to explain glacier flow. In principle, measurements on glacier flow should therefore be capable of telling something about ice creep over very long times, but this is usually not possible in practice because of the very complicated stress systems in a valley glacier.

A floating ice shelf, however, is a much simpler system; it rests on an essentially frictionless bed and spreads under its own weight in a manner which is often uniform for distances large compared with its thickness. In next Monday's *Nature Physical Science*, R. H. Thomas has used data on the spread of ice shelves to investigate the flow law of ice under low stresses. From

laboratory work it had been suggested that the flow became more Newtonian as the stress decreased in this range—that is, the strain-rate tended to be proportional to the stress rather than to some higher power of the stress. The ice shelf data, however, show no such tendency, but seem to support a power law very similar to that found in the laboratory for higher stresses.

Taking into account the mean temperatures of the shelves, Thomas is able to compare the constants in the flow laws derived in these two cases and obtains reasonable agreement. Excellent agreement can be obtained if appropriate corrections are made for the fact that the ice shelves are not completely free to spread in all directions, but are confined at the sides to some extent.

These results are of obvious interest when the laboratory flow law is being extrapolated in the theory of more complex glaciers, but they are also significant as an example of the validity of a power flow law in a solid down to very low strain rates.

POLLUTION

Canadian Conference

ALMOST 1,000 delegates attended the first International Symposium on Identification and Measurement of Environmental Pollutants (ISIMEP) held in Ottawa on June 14-17. The Conference provided a forum for scientists to report on the status of the most fundamental aspect of pollution, namely, that of delineating the problem by identifying and determining the offending substance. Thus the symposium was almost entirely devoted to chemical pollution but the potential contributions of noise and microwave radiation were briefly discussed.

Dr M. W. Holdgate of the UK Department of the Environment, in discussing the need for environmental monitoring, emphasized that effective and economic solutions to pollution problems depended on analytical assessments of the magnitude of the problem. It also had to be realized that local, national or international pollution problems demanded different approaches from the surveillance and monitoring standpoints. Dr W. Foster (US Environmental Pollution Agency) presented the broad forward requirements for combating the extensive pollution and waste problems in the United States, whereas Mr Jack Davis, the recently appointed Canadian Minister for the Environment, outlined his policy in relation to pollution problems in Canada. Mr Davis enunciated a basic abatement principle of "make the polluter pay", and one sensed that he would encounter some rough industrial water in pursuit of this basically laudable goal.

Drs R. A. Chapman and E. Somers (Food and Drug Directorate, Ottawa) in the session on food contamination noted that human food is the end product of a long chain of preparation and processing operations in which there are many opportunities for chemical contamination. Their review highlighted the need for methods, often sophisticated, to maintain a satisfactory level of monitoring. The need for highly sensitive and selective analytical methods, to determine extremely low concentrations of carcinogenic N-nitrosamines in foods, was discussed by Dr J. R. Foreman (Laboratory of the Government Chemist, London). Methods based on gas chromatography with nitrogen-selective detectors coupled with high and low resolution mass spectrometry can yield detection limits of a few parts in 10^9 provided suitable extraction, purification and concentration methods are available. Dr E. Somers considered the problem of heavy metal contamination of food. From analytical data, daily intake levels and existing toxicological evidence, it is concluded that mercury, cadmium and lead con-

stitute the principal human health hazards. The particular problem of determining mercury, both in inorganic and organic forms, was detailed by Dr S. Nishi (Government Chemical Industrial Research Institute, Tokyo) and Dr J. F. Uthe (Fisheries Research Board of Canada, Winnipeg). A result of the work carried out both before and after the recent problem with tinned tuna fish has meant that there is now international agreement on the validity of available methods for mercury determination. Several papers showed the progress in techniques for determining pesticide residues in foods. Dr M. Beroza and colleagues of the US Department of Agriculture, Beltsville, summarized their studies on thermionic and flame photometric detectors for determining phosphorus - containing pesticides and sampling, extraction, clean-up, determination and confirmation requirements for developing multi-pesticide residue methods were reviewed by Drs McCully and McLean (Food and Drug Directorate, Ottawa). Dr A. V. Holden (Freshwater Fisheries Laboratory, Pitlochry), reviewing the

status of methods for determining organochlorine residues in fish, was particularly concerned with the limitations and sources of error in the methods and also the problems associated with less common chlorinated residues.

The chemical complexities of the formation of trace level carcinogenic pollutants were emphasized by Drs Hoffman and Wynder (American Health Foundation, New York) and the photochemical reactions involved in smog formation by Dr R. J. Cvetanovic (National Research Council, Ottawa). The problem of establishing primary gaseous standards at low concentrations was considered by Dr B. E. Saltzman (University of Cincinnati, Ohio) who reported that permeation tubes with lifetimes of over a year could now be prepared. The sensitivity of lichens to atmospheric pollution and their utility for detection of local pollution were reviewed by Dr Brodo (Museum of Natural Sciences, Ottawa) and Drs Schonbeck and van Haut (Landesanstalt für Immusions und Bodenbeurteilung des Landes, Essen).

Excess Cosmic X-rays May Come from Carbon

IN addition to the increasing tally of discrete X-ray sources in the sky—the X-ray stars, galaxies, quasars, and so on—astronomers know that there is a diffuse background of X-radiation against which the discrete sources are seen. None of the many explanations put forward for the background are completely satisfactory, most of them invoking the integrated X-ray emission from one kind or another of extragalactic object—indeed, there seems little to choose between several proposed mechanisms. A problem which may in the end throw further light on this question, however, has arisen in the past year or two; it has been found that at energies around 0.25 to 5 keV the background flux is greater than would be expected from measurements at higher energies. Measurements of cosmic X-rays of energies less than 1 keV are, of course, in their infancy, most measurements during the past decade being at higher energies, and little is known, for example, about how the intensity of the excess radiation varies across the sky. Nevertheless, S. D. Verma of the Louisiana State University has been inspired to draw up an explanation in which the excess of soft X-rays is considered due to line emission from atoms in interstellar space (see next Monday's *Nature Physical Science*). The hypothesis will take its place with other speculation about the origin of this radiation, and will be tested when more detailed data are available.

In brief, Verma writes that interactions between cosmic ray particles and atoms in interstellar—or intergalactic—space will give rise to line emissions which could be the explanation of the soft X-ray excess. Carbon atoms are taken as models, giving rise to the characteristic $K\alpha$ line at about 0.25 keV, although there seems no reason why other elements should not be considered as well, so long as account is taken of their relative abundances.

It remains to be seen whether the improved measurements that will no doubt be available soon will show the excess to be correlated with the expected distribution of carbon atoms in the galaxy, and whether a line structure can be extracted from the spectrum. If not there are other explanations to be considered, and at this stage many astronomers will still need convincing that the excess does not have a more mundane solution in terms of radiations from the Sun which have somehow found their way into the soft X-ray detectors.

Indeed, it is probably still too early also to rule out the possibility that the excess comes from unresolved extragalactic sources, although measurements reported recently by A. N. Bunner and colleagues (*Astrophys. J. Lett.*, **167**, L3; 1971) appear to argue against an origin outside the galaxy. Once again a detailed map of the variation of the strength of the excess with galactic latitude should clinch this question.

EARTH'S ATMOSPHERE

CO₂ versus Aerosols

from our Geomagnetism Correspondent

It has been known for many decades that carbon dioxide in the atmosphere produces a "greenhouse effect"—the trapping of heat in the Earth's lower atmosphere which can lead to an increase in temperature at the Earth's surface. This has often led to the prediction that if man continues to increase the atmospheric content of carbon dioxide by, for example, the burning of fossil fuels, the Earth's surface temperature will rise sufficiently to melt the ice caps and thus inundate many of the world's major cities. The problem with this thesis is that detailed calculations of the effects of carbon dioxide in the atmosphere are hard to find. Thus although it is known that during the past few decades the concentration of carbon dioxide in the atmosphere has increased by about 7 per cent, the precise effects of such an increase are not clear. More to the point, perhaps, the problem of determining the effect of a further increase in atmospheric carbon dioxide is more intractable though even more important. In particular, is it possible that man's activities could produce a "runaway" greenhouse effect whereby in a comparatively short time the Earth's surface temperature would approach, say, that of Venus (700 K)?

According to a calculation by Rasool and Schneider (*Science*, **173**, 138; 1971) such a catastrophe is unlikely because, although the addition of carbon dioxide to the atmosphere will increase the Earth's surface temperature, the rate of temperature increase becomes smaller with increasing amounts of carbon dioxide. Thus even if the quantity of carbon dioxide were to increase by a factor of 8 the increase in surface temperature would be no more than 2 K. The basis of Rasool and Schneider's calculation is a model atmosphere which represents current globally averaged conditions. Details of the calculation cannot be given here; but basically it is a question of computing the outgoing and incoming radiation flux for various concentrations of carbon dioxide in the atmosphere (making, of course, certain simplifying assumptions without which the problem would not be amenable to calculation). The results for the present atmosphere for both incoming and outgoing radiation are in close agreement with experimental data from meteorological satellites; and this gives some confidence that the corresponding calculations for hypothetical quantities of carbon dioxide are likely to give results somewhere near the truth.

Rasool and Schneider have computed the increase in tropospheric temperature required to balance the in-

coming solar flux (for the different concentrations of carbon dioxide) for the case in which the absolute humidity near the Earth's surface is assumed to be constant and for the case where the relative humidity remains constant. The latter produces the higher temperature for any given quantity of carbon dioxide because a warmer atmosphere contains more water vapour; and in the sense that the relative humidity assumption is probably closer to the meteorological truth, the corresponding calculation is probably the better to consider. In fact, although the percentage difference in the temperature increases from the two cases is quite large the absolute temperature difference is small because the increases themselves are small. Moreover, in each case the tropospheric temperature increase tends to reach a plateau as the carbon dioxide content increases. Thus a doubling of the

carbon dioxide concentration produces a maximum tropospheric temperature change of 0.8 K whereas a factor of 10 increase in the carbon dioxide concentration produces a temperature change of 2.5 K. No runaway greenhouse effect is thus possible unless the concentration of carbon dioxide were to become so high that the pressure of the atmosphere were to increase. Because of the pressure effect an increase in carbon dioxide by a factor of 1,000 would produce a runaway temperature rise; but such an increase is unlikely because of the restraining interactions of carbon dioxide with the oceans, the Earth's crust and the biosphere.

The temperature effect of atmospheric carbon dioxide is thus likely to be small even in the long term. In the short term, if the carbon dioxide increases by 10 per cent during the next thirty years

Contact Inhibition and Ribosome Metabolism

THE rate of multiplication of untransformed cells growing in culture is, of course, dependent on the cell density; at high cell densities, in crowded cultures, the individual cells either stop dividing or divide only very slowly, while at low cell densities the cells divide rapidly. And each particular type of cell growing in a given set of culture conditions reaches a characteristic saturation density at which the cells cease to divide. As yet the nature of the chemical signals which bring about this contact or density dependent inhibition of cell division is largely obscure but something must tell a cell when it is in a dense culture and cause it to slow down its macromolecular metabolism. The problem then is to define these signals and also the metabolic processes which they regulate and Emerson reports in next Wednesday's *Nature New Biology* a start in this direction.

Emerson's experiments indicate that both the rate of transcription of ribosomal RNA genes and the turnover of mature 28S and 18S ribosomal RNAs in primary cultures of chick skin fibroblasts are coupled to the rate at which the cells are growing.

He finds that the amounts of ribosomal RNAs in contact inhibited cells dividing every 90–120 hours are about half those in cells dividing every twenty to twenty-four hours and that the rate of net accumulation of ribosomal RNA is about ten-fold less in the contact inhibited cells than in the rapidly dividing cells. After adding a pulse of fresh serum to contact inhibited cells, however, the net rate of accumulation of ribosomal RNA increased within two hours while an increase in the rate of DNA synthesis did not take place

until about eight hours after the serum was added.

Obviously the rate of accumulation of ribosomal RNAs might be regulated either at the level of transcription and processing of the ribosomal RNA precursor molecules or by altering the rate of turnover of mature molecules. In fact it seems that both these processes are coupled to the rate of cell multiplication. For although in contact inhibited cells the rates of formation of 28S and 18S RNAs are some two to four times less than in rapidly dividing cells, the rates of net accumulation differ ten-fold. This indicates that not only are ribosomal RNAs made more slowly in contact inhibited cells than in dividing cells but also that they are less stable and have a shorter lifetime.

Finally Emerson has done a series of labelling experiments in an attempt to decide whether it is the rate of transcription or the rate of ribosomal RNA processing which is reduced by contact inhibition. From measurements of the rate of incorporation of adenosine into ribosomal RNA precursor molecules, coupled with measurements of the specific activity of the pools of ATP and the DNA content of the cultures, he concludes that it is the rate of synthesis of ribosomal RNA precursor molecules, rather than the rate at which they are processed, which is regulated. An analysis of the distribution of labelled AMP along the length of 28S and 18S ribosomal RNAs supports this conclusion. The question now, of course, is what is the mechanism which controls both the rate at which ribosomal RNA genes are transcribed and the stability of the mature RNAs and links these two processes to the rate at which the cells divide.

the increase in global temperature is not likely to exceed 1.0 K. But as Rasool and Schneider point out, even this increase is unlikely to be realized in practice because man's involvement with the atmosphere is more than a question of adding carbon dioxide. Over the period during which the carbon dioxide has increased by 7 per cent the aerosol content of the atmosphere has increased by something like 100 per cent; and the effect of this has probably been to decrease the Earth's surface temperature.

Rasool and Schneider have estimated the magnitude of the scattering and absorption of both infrared and visible radiation produced by typical atmospheric aerosols, using the theory of multiple scattering. Their results show that an increase in the equilibrium dust concentration of the global atmosphere by a factor of 4—which is quite possible within the next century—could decrease the mean surface temperature of the Earth by 3.5 K. Moreover, because of an exponential dependence of the back-scattering by the aerosols the rate of temperature decrease is likely to increase with increasing aerosol concentration.

In spite of the obvious uncertainties in estimating and predicting the effects of carbon dioxide and aerosols in the atmosphere, it seems that, on balance, man's continued pollution is likely to lead to a reduction rather than an increase in global temperature. Thus, far from there being a melting of the ice caps, it is Rasool and Schneider's view that the triggering of an ice age is more likely.

PARASITES

Behaviour of Insects

PROFESSOR D. DAVENPORT of the University of California at Santa Barbara has said that the ultimate aim of any study of animal behaviour is to break down behaviour patterns into a form that can be analysed in physiological terms. The extent to which this has been achieved in the case of parasitic animals and biting insects was one of the problems discussed at a joint meeting of the Linnean Society of London, the British Society for Parasitology and the British Section of the Society of Protozoologists held in London on July 8 and 9.

Dr J. Llewellyn (University of Birmingham) pointed out the difficulties involved in the study of trematodes. The targets of the larvae of monogenean flukes are mostly fish of much greater size and vastly greater speed of movement. Attachment to the host is therefore only possible in stages of the life cycle when they are relatively immobile. Light effects and chemical stimuli from the host, operating over very short distances, are probably the key factors for the parasite. Light is equally important

in maintaining the activity of larval nematodes. Dr N. A. Croll (Imperial College London) described how the normal waning of motor activity can be halted by a five-fold increase in light intensity. By successive increments of this order, involving increases of more than a thousand times over the original level, larvae can be kept on the move indefinitely. This shows that control of activity is likely to be sensory rather than metabolic.

Dr S. Brenner (MRC Molecular Biology Laboratory, Cambridge) asked whether it was possible to "get" the complete structure of a small nervous system. For a nematode a millimetre long, his answer was that one should first describe what is there and how it is connected. Electrophysiology was unnecessary but genetics could be used instead to "ablate" sense organs and to compare the behaviour of mutants with that of normal worms.

The richness of the sensory equipment of biting insects was described by

Dr C. T. Lewis (Imperial College, London). In the antenna of the stable-fly there is a concentration of more than 5,000 sense organs. The use of electrophysiology was essential for their study, but only shows that information of a favourable stimulus has been passed on to the central nervous system. In getting to understand the integration of the latter lay the key to further advances.

The chemical guidance of mosquitoes to their hosts was discussed by Dr M. T. Gillies (University of Sussex) who pointed out that host/vector encounters were dependent on the insect flying into the odour plume of the host. Tsetse flies, on the other hand, tended to follow moving animals. According to Dr A. G. Gatehouse (Imperial College, London) visual factors were primarily responsible for this.

Professor G. D. Nelson (London School of Hygiene and Tropical Medicine) looked at the parasites of man acquired from animals. Those parasites

DNA Duplication *in Vitro*

THE biochemistry of DNA duplication is today almost as great a mystery as ever it was. DNA polymerase I (the Kornberg enzyme) is not essential for the duplication of the *E. coli* chromosome and the same can probably be said of the recently discovered DNA polymerase II, and so with the true DNA duplicase yet to be identified and isolated the field is wide open to all comers. The chief obstacle to progress has, of course, been the lack of an *in vitro* system which maintains the functional integrity of the original replication fork and supports the semi-conservative replication of biologically active DNA at a rate comparable to that in intact cells. These are difficult criteria to meet but according to Matsushita, White and Sueoka's report in next Wednesday's *Nature New Biology* "toluenized" bacteria, bacteria which have been rendered permeable by treatment with toluene, fit the bill.

Last year Moses and Richardson devised this procedure and reported that "toluenized" *E. coli*, in the presence of all four deoxyribonucleotide triphosphates, synthesize DNA rapidly. Sueoka's group, using *Bacillus subtilis* rather than *E. coli* because they could then study the replication of particular marker genes by transformation assays, set out to determine whether the DNA replication supported by "toluenized" cells is indeed semiconservative, whether or not the newly made DNA is biologically active and finally whether replication after "toluenization" proceeds at the replication fork existing before exposure to toluene or depends on new initiation sites.

By allowing the "toluenized" cells to incorporate the density label bromodeoxyuridine triphosphate and then isolating and characterizing the newly made DNA they have been able to prove that synthesis is semiconservative. Moreover, the newly made DNA can be used to transform cells so it is biologically active. But the crucial question is whether this DNA synthesis observed *in vitro* is DNA duplication rather than DNA repair synthesis. If it is the latter, of course, tolenuized cells will be of little use in the search for the real DNA duplicase, but if it is the former, the biochemistry of duplication may at last be accessible to detailed study.

In an attempt to decide this issue, Sueoka and his colleagues first measured the rate of DNA synthesis *in vitro* and *in vivo* and found that tolenuized cells make DNA at a rate ten times less than intact cells. Encouraged by this they exposed a synchronized population of *B. subtilis* cells to toluene and, by measuring the time of replication of selected transformation markers, were able to prove that DNA continues to be made after "toluenization" at the replication fork which existed before this treatment. Both these results strongly suggest that the DNA synthesis *in vitro* is duplication and not repair synthesis. For if it were the latter it might be expected to occur at a slower rate and not exclusively at the existing replication fork. Bottles of toluene will no doubt be appearing on the shelves of every group intent on identifying the enzyme responsible for DNA duplication.

that rely on the ingestion of one host by another for the completion of their life cycles can tip the scales in a predator/prey situation in their favour by making the first host more conspicuous to the second. This was clearly shown by Dr J. C. Holmes (University of Alberta) who described how parasitized freshwater shrimps swim to the surface and attach themselves firmly to reeds and floating debris that are more efficiently searched by ducks than the muddy bottom of a pond.

NUCLEIC ACIDS

New Exchange Rates

from our Molecular Biology Correspondent

THE trouble with hydrogen exchange is that although the results are full of information, it has often been unclear what they mean. Several interesting ideas have nevertheless emerged: the relatively slow exchange rates in DNA, for example, compared with mononucleotides, gave substance to the notion of a "breathing" effect in the double helix, involving the transient melting of units of a few base pairs. It was assumed that the reluctant hydrogens in the double helix were only those involved in hydrogen bonds between base pairs, but because of the difficulty in extending the rate plot to very short times the number remained unknown.

That any useful measurements were feasible within the time-scale of exchange in nucleic acids was due to new methods devised by Englander of achieving rapid separation of the nucleic acid from tritiated solvent. By a number of innovations of technique Hanson has effected a diminution in separation time by another order of magnitude, and in a paper in *J. Mol. Biol.* (58, 847; 1971) he now describes his new results on the tritium exchange kinetics of DNA. Using short columns of 'Sephadex' to separate the DNA from the tritiated water, with forced elution under pressure, the extraneous counts can be separated from the sample in about 6 s. The essential benefit of extending the data to these short times is that the lower limit of the intercept for the number of unexchanged hydrogens at zero time can be raised. With the previous data and with times down to 100 s, the zero-time extrapolation looks to be heading for a value close to the number of protons in Watson-Crick pairs, for example, 2.4 per base pair for calf thymus DNA. Hanson's data show that this extrapolated value is much too low and that a value of 3.8, corresponding to all the N-H protons in the base pair, seems closer to the truth.

An A-T base pair has three exchangeable protons and a G-C pair five, and indeed the higher the G-C content of

the DNA, the higher the value at which the rate-plot approaches the zero-time axis. For the alternating polymer, poly d(AT), the value of two protons is again much too low, whereas three looks altogether convincing. Two-stranded poly(dG)-poly(dC) presents difficulties of preparation, but Hanson quotes unpublished data that indicate a value of five hydrogens exchanging at measurable rates. Single stranded polynucleotides, such as poly (A) exchange completely in an immeasurably fast time. It seems then that exchangeable hydrogens in the double helix grooves are protected against exchange. It is probably scarcely profitable to speculate on the mechanism of this retardation—it may or may not involve the structure of water in the hydration shell, the local dielectric constant, or yet other effects. At all events these results provide a new basis for the interpretation of exchange data for nucleic acids in various circumstances. The view still stands that exchange occurs by way of an unpaired state, but that the "breathing" frequency is too great to be rate-limiting. A possible explanation for the spectrum of exchange rates observed in DNAs is that there are two or more "open" or "deformed" states of short duration, offering varying degrees of accessibility to the solvent. Hanson points out the possibilities which rotation rates of amino-groups

about the N-C bond within the lifetime of the "open" state offer for determining the equivalence or non-equivalence of the two protons of a hydrogen-bonded amino-group, and he also notes the environmental differences between G-C protons in the wide and in the narrow groove.

An empirical but useful application of the tritium exchange method has been to the study of ribosomes. Here both proteins and RNA have exchangeable protons, some of which are replaced quite slowly. When the subunits are dissociated there is evidently a large conformational disturbance, for rapid exchange at once ensues. Chuang, Silberstein and Simpson (*Arch. Biochem.*, 144, 78; 1971) now report that the exchange rate of 70S *E. coli* ribosomes is detectably modified at the translocation step. The addition of translocation (G) factor and GTP causes a change in the exchange-rate profile, which is not observed when an inactive GTP-analogue is used, or when the translocation inhibitor, fusidic acid, is included. Chuang *et al.* conclude that translocation is accompanied by far-reaching conformational upheavals in the particle, and they permit themselves the fancy that such a distortion of the ribosome cranks the particle along the messenger, while translating the peptidyl-tRNA from the aminoacyl to the peptidyl site.

Superheavy Elements

ONE of the most exciting aspects of nuclear physics in the past few years has been the realization that there might be stable nuclei with a nuclear charge of $Z \approx 112$. The first experimental results that purported to show the existence of such nuclides were obtained by a team based at the Rutherford High Energy Laboratory and published in *Nature* earlier this year (A. Marinov *et al.*, 229, 464; 1971). They claimed that the result of their experiment was not inconsistent with element 112 having been formed when a tungsten target was bombarded with 24 GeV protons. The proposed mechanism for formation of the superheavy element was based on an energetic tungsten nucleus being given enough energy by the proton beam to make it possible for it to react with a stationary tungsten nucleus in the target, and thus lead to a reaction that would form element 112.

This proposed reaction mechanism was soon recognized as the weakest part of the interpretation of Marinov *et al.* and the feeling was that this process would not have a sufficiently large cross-section (that is probability of occurrence) for the reported amount of element 112 to have been formed. This

argument is put on a quantitative basis in next Monday's *Nature Physical Science* by P. K. Kabir, himself from the Rutherford Laboratory, and J. S. Trefil of the University of Virginia in work carried out at Virginia while Kabir was on leave. Their calculations give a cross-section for formation of element 112 that is 10^9 times smaller than the value assumed by Marinov *et al.* to explain their results.

This could possibly be the last word and some explanation, other than the production of element 112, would have to be found to explain the effects observed by Marinov *et al.* This does not seem to be the case as Dr Christopher Batty, one of Marinov's colleagues and the coordinator of the work at the Rutherford Laboratory, in a reply published with the article of Kabir and Trefil points out that the calculations are not in agreement with experiment and he cites two recent publications that show the measured cross-sections for the production of fast heavy nuclear fragments to be much greater than calculated. The stage is set for another round of discussion of the work of Marinov *et al.* and the efforts currently under way to confirm the existence of element 112 will continue unabated.

Science Policy in the Developing Nations

R. S. BHATHAL

Physics Department, University of Singapore

South-east Asia provides an illustration of the need for changes in fundamental attitudes towards education, science and technology if developing countries are to formulate their own science policies, with central organizations to carry them out.

It is surprising that although the governments in South-east Asia have from time to time made statements about the role of science and technology in their programmes of national development, only about half of them have either a ministry of science and technology or a national research council to control and direct science policies. On the other hand, the academics at the universities are still arguing about the merits and defects of planning for science, when the great debate of the 1930s between the planners and non-planners has been fought and done with in the western centres of intellectualism. In the developing nations a further difficulty arises because the whole issue is coloured by the political leanings of the debaters in question. Today, however, hardly anyone who moves in the "right circles" would deny that science cannot be planned, at least within the restricted notion of planning. Most planners of science policy are aware that the results of scientific research cannot usually be predicted, but they would admit certain guidelines could be prescribed for future development. The question of choice is no very new concept to the scientific establishment because choices¹ are made and will always be made in any scientific undertaking, and the question of allocating resources or stopping a particular line of inquiry is always being considered at the research establishment level.

Furthermore, economists in South-east Asia have on the whole tended to see economic growth within the rather restricted notion of increases in national income, and as such their parameters have been capital accumulation and labour productivity. This is not to say that they are unaware of the influence of science and technology on economic growth, but, rather, that they have still to account for it in explicit terms. Just as in the debate on planning, so also in economics there has been a time lag in the appreciation of science and technology in economic growth by those concerned with these studies.

To put the findings of science and technology to effective use for the purposes of economic and social development, countries in this region must formulate science policies. It is well known that the political and foreign affairs of a nation can be scrutinized intellectually and, within the limits of these kinds of studies, it is possible to provide a general framework within which the political affairs of a nation can be run. It is not surprising therefore to hear of a "foreign policy" or an "economic policy" for the nation. Although the concept of science policy is new, it should be possible, by analogy to the foreign policies or the economic policies, to talk of a science policy for the nation.

Science Policy

The term "science policy" is rather ambiguous, for it tends to imply a concern for the needs of science only, with no regard to its impact on the other sectors of the economy. A well formulated science policy is concerned not only with the needs and development of science, but also with the application of advanced technology to the improvement of the social conditions of the country. A brief analysis of the various fields which interact with science at the national level suggests that science policies in the developing nations should be concerned with the following aims: (a) to train and educate technicians, engineers and scientists, so that a proper scientific manpower level is maintained; (b) to make the general public aware of the social and cultural benefits of science and technology by arranging talks, forums and exhibitions; (c) to support research which does not directly increase the growth of the economy of the nation, for example, biomedical and sociological research; (d) to support and encourage basic and applied research in government, industrial and university laboratories for the long term development of the scientific resources of the country, and (e) to examine the need for local industrial research and development for the short term needs of the country.

To implement such aims of science policy it is necessary to set up a central organizational structure with the proper channels for action. This entails the establishment of either ministries of science and technology or national research councils to advise the governments on the course of action they should follow.

In Malaysia, for example, there is no central body or national science policy to look after the affairs of science or its implications for social development. Most decisions on science are made in an *ad hoc* manner or pronouncements are made regarding the role of science in the hope that they may catch the ear of some members of the government. In this respect Singapore is better off in that it has set up a ministry of science and technology for making national science policy decisions. The initial formation of the sub-committees of the Science Council in Singapore well illustrates a fault which is common among developing nations; they import the administrative structure from the more developed nations without the accompanying activity² which is essential to the success of any plan. The Science Council of Singapore was formed in 1967 under the Science Council of Singapore Act, 1967³, to make reports and recommendations to the minister on: (a) scientific and technological research and development; (b) the effective training and utilization of scientific and technological manpower in Singapore; and (c) the establishment of official relations with other scientific organizations.

To examine the problems generated by these recommendations, four sub-committees were appointed to deal with engineering, physical sciences, social sciences and natural resources and their utilization. Thus the administrative machinery was set up, but no action followed. It is, however, to the credit of the policy makers that they realized this early in the development of the Science Council and immediately took remedial steps. Instead of having these sub-committees

it has proved more useful to establish committees as the need arises, and the structure of the committees has been changed; they are now set up to solve specific problems.

Scientific Manpower

One of the basic aims of any science policy in the developing nations is to ensure the adequate supply of trained scientists, engineers and technicians, so that the scientific manpower level is always kept in balance. Too often in the developing nations this aspect of the manpower planning programme is not given the serious attention that it requires, and it is not surprising that mistakes in the educational system may only become apparent after six or ten years, so that the whole process of re-educating the citizens or restructuring the educational system has to be carried out. This has serious drawbacks as far as scientific and technical manpower is concerned because it eventually delays the introduction of science-based industries. Apart from this, some of the countries have geared their educational programmes to produce not technical personnel but pure scientists, in the hope that this would produce the desired change in the social and economic conditions of the country. No one questioned the kind of scientists needed, with the result that there was an overproduction of pure scientists at the expense of engineers and technicians.

Of course decisions about the supply and demand of trained scientific and technical manpower impinge on the educational and social policies of a country. In Singapore, for example, the withdrawal of British troops, and a realization by the leaders of the country that long term prosperity could not depend solely on the *entrepot* trade, led to an intensification of the industrialization programme which began in the early 1960s. A recent survey, however, showed that in 1968, 86% of pupils were enrolled in academic-type secondary schools, while 14% were enrolled in vocational and technical schools⁴. Here was glaring disparity in the educational system when the country was embarking on a serious industrialization programme. Remedial measures have now been taken, and it is hoped that by 1972 one-third of secondary school leavers would have passed through the technical/vocational stream. The full impact of this change will not be felt for a few years as there is always an inherent time lag in any educational reform. But although there has been a great effort to increase the output of technical personnel, policy makers have to contend with the inherent "white collar mentality" in Singapore society. Not only is manual or technical work still frowned on, but the monetary remuneration is not very attractive. It has, however, been suggested that one way to break this white collar mentality is to increase the wages of the labouring and technical classes as opposed to the clerical workers. But the issue is complicated by the question of security and working conditions of this class of workers. Thus the problem goes beyond science policy decisions and begins to look more like a problem that has to be dealt with in part by sociologists. And so there is a need for sociological research.

One has to tackle problems that involve the whole of society before any meaningful progress or change can be brought about. These problems are not insurmountable but they require policy makers to be aware of the many aspects of development policies. Most policy makers and their executors in the developing countries, however, tend to be single discipline honours graduates, who are not likely to see the boundaries where their disciplines merge into other disciplines. Many of the problems of developing nations require an inter-disciplinary approach, which again highlights the problem of educational policy and its implementation. To implement educational reform, however, one must change old habits, and this is very painful to the established order. Changes in the degree structure are now in progress in the universities of Singapore, but the full impact of these changes will not be felt for a few years yet.

Need for Engineers

During 1964–1968, the institutions of higher learning in Singapore produced 171 professional engineers and 1,718 scientists. On the other hand, in Japan in 1962 there were seven engineers to one scientist. With the expected increase in the number of firms which are setting up their factories in Singapore, the need for engineers has been felt acutely by industry. Most engineers are needed in the manufacturing, construction and utilities sectors of the economy. It has been estimated that about 3,000 engineers will be required between now and 1980. But, although there has been much discussion about the need for more engineers, the question of what kind of engineers are needed has still to be answered. Furthermore, with the possibility of an increase in the projected demand for engineers because of increases in military requirements and the possibility of Singapore becoming a regional consulting centre, it is unlikely that the supply of engineers will match the demand at the existing rates of production.

A question that might have important implications for the growth of the scientific community of the nation is that regarding the total number of scientists and engineers which a country needs before it can be said to have passed the scientific threshold. What the exact size should be is difficult to gauge. In addition to the size of the scientific communities, it is also important to look into their nature to see whether they have taken root in the country or are chiefly composed of foreign staff who will sooner or later leave it. This is important if one is concerned about creating a viable local scientific community.

New Priesthood

Another aim of science policy in the developing nations is to make the general public aware of the cultural, political and social benefits of science. Most developing nations have pre-scientific cultures and the great clashes between science and religion, and the more recent one between science and politics, have not occurred. Consequently there is no real involvement of the general public with science, so that science is regarded as a collection of gadgets, and scientists are "knob-turners" and not men with ideas. These attitudes can be changed by, for example, exhibitions, but the exhibits must be chosen so as not to instil a sense of awe at the gadgets of the new priesthood. Exhibits showing some of the physical principles involved are likely to be more educational than models of a power station or a rocket or pictures of men walking on the Moon. Of course the latter are useful, but not from the point of view of showing the physical principles involved.

The popularization of science could be more effectively propagated through talks and forums, but this requires a central organizational structure, such as a body for the advancement of science. Although many developing countries have bodies for the advancement of science or national academies they tend to be rather ineffective. Another means of popularizing science, which may prove to be very useful in the developing nations, is the introduction of general science courses at universities for arts students⁵. These people usually go on to form the administrative backbone of the government and commerce, and an exposure to science should help them to appreciate the needs of science. Furthermore, as an elite in a developing nation they will be in a better position to propagate the concepts and methods of science. At the University of Singapore a general science course has been introduced into the arts curriculum in the hope that those who occupy administrative positions will be able to appreciate and not retard the introduction of science-based industries in Singapore. Another equally effective method of popularizing science is through science magazines which are specially written with the intellectual and scientific level of attainment of the consumers in mind. There are, of course, a large number of popular science magazines on the market which are produced by the advanced nations; and although on sale in the developing

countries they are usually much too expensive for the people living in these parts of the world. There are two ways of overcoming this problem. The first is to use a method which has been successfully employed in the production of cheap editions of expensive American science textbooks. This method could well be used with profitable results for the production of cheap Asian editions of popular American or European science magazines. The second method would be to produce the magazines locally. However, the problem here is that they will have to compete with the international science magazines not only in content and attractiveness of layout but also in having a continuous supply of material from local contributors. This is probably a good venture for local societies for the advancement of science if they can muster enough talent in the field of scientific journalism.

A further aim of science policy in the developing nations is to support and encourage research in government, industrial and university laboratories which either does or does not help in the growth of the economy of the country. The decision to support and encourage research in the developing countries must come directly from the government, which is always the chief source of funds. Money spent on research in the developing countries seldom, if ever, helps in the economic growth of the country; the effect is indirect.

Science Budget

Table 1 shows the allocation and distribution of financial resources to science in Singapore derived from a recent Unesco survey⁶. The figures for the budget include salaries

Table 1 Science Budget for Singapore (1965–1966)

Fields of activity	Budget (\$)	Percentage of budget
Physical and chemical sciences	156,738	4
Biological and agricultural sciences	1,568,549	40.5
Medical sciences	420,658	10.9
Technical and engineering sciences	1,205,769	31.2
Industrial science	190,000	4.9
Meteorology	329,000	8.5
Total	3,870,714	100

and wages, local operating costs and capital expenditure for the year 1965–1966. The budget figures have been converted at the US dollar exchange rates existing at that time. It is not clear from these figures how much money is actually spent on research, but they give an idea of how much is spent on science in general and how it is distributed between the various fields of activity. Ninety-three per cent of the budget comes from the government, which decides how it will distribute this money. The other 7% of the budget is derived from private donations or the activities of the various departments. Thus none of the departments which undertake research or testing can exist solely on their research activity; this is probably true of the other developing nations. There is no separate science budget in Singapore. Although the figures for the distribution of finance (Table 1) are rather *ad hoc*, they give an idea of the relative importance attached to the various fields in science. A large proportion of the budget is spent on the biological and agricultural sciences, chiefly on the Government's Central Research Station which does research on livestock, agriculture and fisheries. This is justifiable because Singapore is an island state with few natural resources, thus requiring self-sufficiency in these fields. Because of the rapid increase in constructional projects and the speed of the industrialization programme, 31.2% of the budget is spent on the technical and engineering sciences, most of it being used by government institutes. Meteorology has been allocated 8.5% of the budget because Singapore is an important air and naval communications centre. The physical sciences have been rather neglected, but in the interests of the long term development of scientific resources this will have to be revised

and more attention paid to the needs of the basic sciences. As in all developing countries, most research in Singapore is carried out in the universities. As is often pointed out, research acts as a catalyst in university education, and recent studies indicate that major contributions to economic growth come from "residual factors" such as education and research⁷. Although these studies were concerned with advanced countries, they indicate the path that developing nations could well follow, if they are considering scientific objectives on a long term basis.

The collection of basic data about the scientific resources of a country, as Unesco has started, should be part of any science policy programme, for it not only provides useful information but serves as a guideline for the allocation of finance.

Transfer of Technology

For their short term needs the developing nations must decide whether to invest in their own industrial research and development, but there is often not only a shortage of technical and scientific personnel, but also no research environment. Japan, for example, spends less than the United States on research and development, but its annual percentage growth of national product per man is much higher than that of the United States⁸. This has been made possible by Japan's wisdom in buying technical know-how from the industrialized countries, particularly during its early development, and even now in certain branches of industry.

Here is a lead for the developing nations to follow. If they can achieve a relatively stable political climate, they can and should attract foreign subsidiary science-based companies to set up factories in their countries. The choice should be based on attracting labour-intensive industries, for this will help to solve some of the acute problems of unemployment. For example, Singapore has managed to attract and set up electronics and ship-building industries. The research and development costs of the subsidiary companies will invariably be covered by the parent companies. They are in a sense "cheap labour economies" but they will help in the transfer of technology from advanced to developing nations. Rather than talking in terms of research and development in the developing nations we should begin to consider the problems of the transfer of technology to these nations, for this approach may be more useful in starting an economic and scientific development. A programme devised by the Science Policy Study Unit at the University of Sussex, to study the diffusion and transfer of technology in Thailand will perhaps throw further light on this problem, and the results should prove very useful for other developing nations. It would be interesting to make a comparative study of this aspect of science policy between the countries of South-east Asia.

It is becoming increasingly apparent that the many problems facing the developing nations in science and technology have to be scrutinized in much the same way as the problems involved in the formulation of an economic policy. The onus for the development and cultivation of science rests on the governments and the people of the developing nations themselves, because no amount of outside help will effectively solve the problems they face unless they make a determined effort themselves.

¹ Maddox, J., *Minerva*, II, 2, 141 (1964).

² Aron, R., in *Decision Making in National Science Policy*, Ciba Foundation Symposium (Churchill, London, 1968).

³ *Ann. Rep. Science Council of Singapore 1967* (Government Printing Office, Singapore).

⁴ Bhathal, R. S., *New Sci.*, **44**, 514 (1969).

⁵ Bhathal, R. S., *Technology and Society*, **6**, No. 2 (1970).

⁶ *Research Facilities in Science and Technology in Asia* (Unesco, 1968).

⁷ *Fundamental Research and Policies of Governments* (OECD, Paris, 1966).

⁸ Williams, B. R., *Investment and Technology in Growth* (Statistical Society, Manchester, 1964).

"Self-recognition" in Colonial Marine Forms and Flowering Plants in relation to the Evolution of Immunity

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Compatibility in colonial tunicates and coelenterates and self-incompatibility in flowering plants are primitive examples of "self" and "not-self" recognition which are not analogous to the immunological processes of vertebrates and which shed light on the way in which adaptive immunity may have evolved.

THERE is a growing tendency to regard the evolutionary origin of adaptive immunity, as manifested in ourselves and in the standard experimental mammals of the laboratory, as being related to something other than defence against pathogenic microorganisms. In 1959 Thomas¹ suggested that there were two mammalian phenomena which might be relevant to the understanding of immunity and its evolution—pregnancy and malignant disease. Both suggestions have subsequently been discussed extensively and the relationship of the foetus as a mass of antigenically foreign cells embedded in the maternal tissues to the mother's immune system has provoked much interest and experimentation^{2,3}. Similarly, the antigenic quality of a large proportion of autochthonous tumours became one of the central themes of recent oncological research⁴⁻⁷. I have written extensively⁸ on the concept of immunological surveillance, which postulates that antigenic patterns abnormal to the genotype which arise by somatic mutation may provoke an antigenic response and play a part in preventing or delaying the appearance of malignant disease.

All metazoan forms must have a capacity to inhibit the multiplication of bacteria and other potential pathogens in their tissues and in that sense they are possessors of an immune system. For obvious anatomical and functional reasons, if we are to consider the evolution of the mammalian immune system, we must confine ourselves to the vertebrates and their immediate ancestral forms. Recently there have been intensive studies of sea lamprey^{9,10} and hagfish¹¹⁻¹³ as representative modern cyclostomes, the most primitive extant vertebrates. It is probably a fair summary of the results to say that homo-graft rejection is well shown; there are other T-type (cell

mediated) responses and restricted and weak antibody responses. The immunoglobulins have typical vertebrate characteristics but they are in very low concentration¹⁰.

Studies of Colonial Tunicates

No immunological studies have yet been made to my knowledge of the protochordates and the work of Oka¹⁴ on the genetics of compatibility in colonial ascidians has not been considered previously for its immunological implications. Here I shall explore the possibility that Oka's studies of colonial tunicates (*Botryllus*) and the less extensive studies of Theodor¹⁵ of the anthozoan (*Eunicella*), also a colonial form, may throw light on primitive types of "self and not-self" recognition from which adaptive immunity may have evolved.

The colonial ascidian *Botryllus* produces star shaped colonies, each ray being an individual with its own inlet and outlet pores for seawater circulation and feeding, but with a common vascular system ending in a series of peripheral ampullae. The colony is initiated by a larva that develops from a fertilized ovum, but once the larva has settled down it buds off new individuals to form the colony. Each colony is therefore essentially a clone of genetically identical units. As might be expected, therefore, if colony I is divided into two and the parts allowed to grow separately and then brought into contact, the two daughter colonies fuse together and reconstitute a single colony. If, however, an unrelated colony II of the same species is brought into contact with colony I there is a positive rejection and a barrier of necrotic material develops between the two colonies. Finally, if another compound ascidian of the same general type, *Botrylloides*, comes into contact with *Botryllus* I or II, nothing happens. One will grow over the other as if it were just an inert attachment surface. Clearly, therefore, there is recognition of foreignness within the species and recognition must always be the basic phenomenon of immunity.

Sexual reproduction of *Botryllus* is dominated by mechanisms to avoid self-fertilization since the organisms are hermaphrodite, liberating both sperm and ova into the environment. To summarize Oka's work, it is convenient to accept his assumption, which is validated by much preliminary work, that fusion or rejection between colonies depends on a single locus with many alleles which can be referred to as recognition genes. All natural colonies are heterozygous and can be represented as AB, CD and so on. At the margin of each colony or regenerated sub-colony there is a row of ampullae (with a single cell wall) the contents of which are part of the common blood

circulation of the colony. It is the reaction of contact between these ampullae which determines the gross character of the interaction between two colonies. When two parts of the same AB colony are brought together again, the walls of adjacent ampullae fuse and a lumen is developed to extend the common circulation. When an F1 generation is produced, the progeny from an AB $\times$ CD cross will be of four different types: AC, AD, BC and BD. Any two sets of these which have a gene in common—AC/BC, AC/AD etc.—will show the same type of fusion and integration of the circulation. Where there is no common gene—AC/BD, AD/BC—the ampullae in contact undergo necrosis, producing a barrier between the two colonies.

The other feature of interest concerns cross-fertilization. These organisms are hermaphrodite and there are special arrangements to prevent self-fertilization. The gametes which are, of course, haploid are A or B from AB and C or D from DC. Self-fertilization does not occur presumably because of the layer of follicular cells which surrounds the ovum. The nature of this structure is not elaborated in Oka's review¹⁴ but the cells surrounding an A ovum from an AB individual are presumably diploid and AB, and prevent the entry of either an A or a B spermatozoon. Any other type of spermatozoon, such as C or D, encounters no obstruction.

Other Colonial Forms

Both phenomena seem "designed" to ensure the greatest degree of heterozygosity in all viable colonies. It is virtually impossible for a homozygous colony to be formed and, at the same time, the situation cannot be muddled by the fusion of unrelated colonies. Both seem to produce colonies which are at a disadvantage for survival, although it is not easy to see just what the disadvantage is. Oka points out that the situation is directly analogous to the self-incompatibility of angiosperms such as plums, but it is even more analogous to the findings of Theodor¹⁵ on some colonial coelenterates (Anthozoa). It may well be of importance for survival that colonial marine forms should be able to distinguish their own colony from another. In the sea-fan *Eunicella* the colony has a branching form supported by a firm sclero-protein axis on which there is a continuous sheath of living cells from which the polyps derive a dense efflorescence. Taking 25–30 mm segments of the branches, Theodor denuded a central 2 or 3 mm portion to expose the rigid non-living axis. Two such preparations were combined so that the two denuded areas formed the meeting point of a right-angled cross. When such preparations were maintained in suitable aquaria, regrowth eventually covered the denuded regions. If both segments were from the same colony all four growing surfaces fused without any sign of discontinuity. When one segment was from colony A and the other from B, A and B tissues failed to fuse and a clear line of demarcation was visible. When two different genera, *Eunicella* and *Lophogorgia*, were placed in contact there was a damaging interaction in the form of the development of "blistery" tissue along the surface of contact.

Self-recognition in *Botryllus*

As far as the evolution of adaptive immunity is concerned, it can be assumed that the phenomena Oka described for colonial ascidians, and probably also those of Theodor in the Anthozoa, facilitate the achievement and maintenance of heterozygosity. In the better studied system of Oka, the aspect of immediate interest to the immunologist is the destructive interaction between cells lacking appropriate common alleles of the recognition genes. This, however, has no more than a superficial resemblance to the cytotoxic action of sensitized lymphocytes on a larger cell. There must be three distinct sets of recognition phenomena. First, a somatic cell in contact with another recognizes that they possess at least one common recognition allele and as a result the necrotic response² is inhibited. Second, a somatic cell in contact with another which

has no common specific allele but is otherwise genetically similar (of the same species) can recognize this conspecificity in the sense that it induces mutual necrosis. This is not seen when wholly unrelated forms make contact. Third, a sperm (haploid) recognizes the presence of the same allele by a gene product present in the periovular cells and cannot fertilize such an egg.

The concept of recognition is so important that these three examples justify discussion in some detail. The essence of the matter is that organism tissue or cell A comes into contact or other definable relationship with two or more generally similar entities, X^1 , X^2 , X^3 and so on, and in one instance (say, with X^1) a demonstrable and reproducible reaction occurs which is not seen with X^2 , X^3 etc. A is then said to be capable of positive recognition of X^1 . Sometimes, as in the case of *Botryllus*, the individual reaction by which recognition of one character as against "any others" is shown takes the form of an inhibition of necrosis or some otherwise inevitable response. The crux of the matter is that recognition is only meaningful when it is a recognition of one out of many alternative candidates. An antibody reacts visibly with only one of a thousand antigens. In the *Botryllus* examples we must postulate that there is a positive recognition of the presence of a common allele in the somatic cell genome, a positive recognition of conspecificity by the mutually damaging reaction and a positive recognition by the sperm that the follicular cells of the ovum have the same R allele, since it can fertilize "all other" combinations which lack that allele.

Nature of Recognition

All those positive recognitions are conventionally and almost certainly correctly interpreted as representing specific union, reversible or irreversible, between chemical groupings on the surfaces of the interacting cells. There are possible ways by which like chemical groupings can recognize each other as exemplified in the growth of any crystal, but where proteins are concerned this seems to be a cumbersome approach inappropriate for the extremely heterogeneous micro-environment where all recognition reactions occur. All immunologists, every biochemist concerned with enzyme-substrate relationships and every pharmacologist interested in drug-receptors implicitly accept the axiom that all such specific ("recognizing") reactions are based on sterically complementary (+/–) chemical patterns. We are bound to do the same here, but to do so brings us immediately up against a genetic difficulty whose solution may be of first rate importance for immunological theory. Two of the examples of recognition seem to be recognition by cell X that cell Y has an allele identical to its own. If we exclude, as we must, any suggestion that recognition involves interaction between nucleic acids, this seems that any mutation to a new allele must in some way produce corresponding but complementary changes in both + and – patterns of the mutual recognition sites. It is extremely difficult to conceive any way by which information in a DNA sequence (A) can provoke the formation of another DNA sequence which will code for a complementary three-dimensional structure that will react specifically with the product of A. It seems more promising to consider the situation where the + and – complementary receptors are controlled by distinct genes. There is much to be said for an *ad hoc* assumption that both + and – types of recognition gene are derived by duplication from a common progenitor gene, but they would require to be capable of independent mutation. We have to assume that a mutation in, say, the + gene cannot be expressed until a corresponding mutation arises in the – gene. If such a process were to function there would have to be a high mutation rate in genes, a situation which has obvious relevance to the genetics of immune pattern and to Jerne's ideas¹⁶ of a special relationship between the potential antibody patterns transmissible by the germ line and histocompatibility antigens.

All that I have been discussing in regard to the colonial

ascidians is solely concerned with germ line genetics. Each cell is regarded as expressing appropriately the instructions of the germ line genome. Somatic genetic changes come later in the story.

The situation in *Botryllus* is represented schematically in Fig. 1. Here it is assumed that at each surface of contact with an adjacent cell there are + and - receptors for the two relevant alleles of each heterozygous diploid colony. A positive receptor is shown as a knob, a negative one as a depression. By hypothesis, +/− union stabilizes the two adjacent cell surfaces, and in the absence of any +/− unions mutual disorganization with liberation of damaging products takes place. It is inexpedient to attempt any biological explanation of this phenomenon except to point out that in all larval-adult metamorphoses there is extensive cell destruction in which some such processes must be involved and also that similar cell damage is seen in all severe T-D immune reactions in mammals.

Relation to Immune Response

At most this can only be a foreshadowing of the adaptive immune system of vertebrates. There is, for example, no hint as to how the capacity to recognize foreign qualities of con-specific cells evolved and there are manifest differences of the tunicate system from that subserving tissue integrity (the T-immune system) in vertebrates. Like every biological invention the utilization of steric complementarity involves both the informational store in the genome and the protein molecules needed for its phenotypic expression. There arises immediately the outstanding problem of how mutation and other informationally random genetic processes can produce complementary structure in the products of two distinct genes. Yet once that evolutionary discovery had been made it could provide the raw material from which in colonial organisms means could have evolved to ensure the genetic integrity of the colony and to avoid the long term dangers of self-fertilization and inbreeding. A continuation of biological invention along the same general path could in principle lead to the construction of a vertebrate immune system. For this to happen, there are three requirements: first, mutual recognition systems would have to be elaborated and certain cells differentiated to specialize in recognition and in the local damaging response; second, recognition functions of such cells would have to become progressively specialized until there existed a wide diversity of immune patterns, each limited to a single clone, and third, such "immunocytes", in certain conditions of specific stimulation, would have to be able to take on the blast form and proliferate.

It is probably unwise to attempt to imagine the various steps by which such changes could be made. One can foresee a period of great research activity in these fields of tissue fusion and rejection in invertebrates and protochordates during the next decade. Undoubtedly a variety of intriguing phenomena will be uncovered, differing from group to group. Some may be further along the road toward adaptive immunity than the colonial ascidians. Much more extensive comparative studies are called for and in due course analysis of the results should allow a clear evolutionary history to emerge. Whatever form that history eventually takes we can be certain that gene duplication (gene expansion) plays a major part, and that progressive specialization of cell function and phenotypic restriction will be as conspicuous as it is in all other organs and functions.

Viviparity and Parasitism

Evolution is much more than an expansion and specialization of rudimentary functions present in the form we choose as a starting point. It is a process of modification for more effective survival of populations. Major evolutionary changes probably always mean that a major danger to survival has appeared and must be overcome or that a new ecological niche has arisen to be occupied by species which can first make the necessary adaptations. Often, of course, the two reasons overlap.

A year or two ago I suggested that when free swimming marine protovertebrates ancestral to cyclostomes first appeared it is conceivable that one of the early results was the emergence of a new ecological niche, survival by parasitism on one's own kind. The stimulus to develop this point of view was the immense ecological effect of the entry of the sea lamprey into the great lakes of North America around 1930¹⁷. Lampreys are cyclostomes that live by blood sucking—one lamprey can kill 14 kg of fish per annum—and there were countless streams running into the lakes which were suitable for larval development. Lamprey proliferation reduced the economically significant fish species to such a level that the lakes' fishing industry was destroyed. The subsequent ecological history of the Great Lakes is of great interest but not relevant to my theme, which is that with the entry of an effective predator-parasite in the form of a cyclostome a major ecosystem was wholly changed. If free swimming protovertebrates in Cambrian seas represented the current evolutionary success, a wide "radiation" into new ecological niches must have begun immediately. There is evidence that lamprey-like forms were present in the Silurian¹⁸, presumably living like modern lampreys but necessarily on other cyclostomes until fishes had evolved.

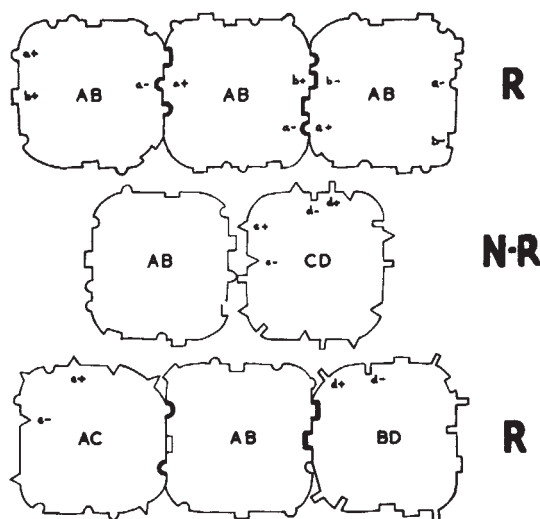


Fig. 1 A diagram to suggest the type of +/− relationship between recognition units that is postulated for *Botryllus*. A positive receptor is shown as a projection, the corresponding complementary negative receptor by a depression of the same shape. Where there is a reciprocal relationship (R) allowing receptor union (heavy line), the relation is stabilized. In its absence (N-R), fusion is impossible.

Against this background I asked¹⁹ whether parasitism of a cyclostome by its own young or by related species might not provide an urgent demand for the development of a more refined capacity to recognize the difference between self and not-self. This could be the evolutionary stimulus needed to set the construction of an adaptive immune system on its way. In the light of what has been published since, I should now be inclined to state the possibilities slightly differently. What I failed to comment on was the significance of viviparity which appears in many lower organisms including tunicates and fish as well as in mammals. When a living embryo is nourished in the tissues of the parent the situation is barely distinguishable from parasitism, and oscillation between a controlled situation and uncontrolled parasitism must have occurred not infrequently. There may well have been a special predilection for viviparous larvae kept under control in their own parental environment to make an early switch to parasitism on a similar type of organism.

Comparison with Homograft Reactions

At this stage it is necessary to attempt a formal comparison at the genetic level of what is observed in the colonial ascidians and what holds for homograft reactions in mammals. If in both the simplifying assumption is made that histocompatibility is determined by alleles of a single gene, we can consider the results of matings between heterozygous animals whose genotypes are AB and CD, AB being used for the female in each case. For both types of animal the F1 generation will contain the genotypes AC, AD, BC and BD. In *Botryllus* all possible offspring will fuse (be compatible) with the parent. In a mammal, skin grafts from any of the offspring will be rejected. The evolutionary problem is how to pass from the first condition to the second.

First, it is necessary to establish a process by which recognition can have a second type of sequel. Instead of serving only as a means of stabilizing cell interaction at contact surfaces, recognition must under other circumstances be followed by a damaging liberation of cell products. Second, complementary patterns must be provided which can recognize not only gene products which can be produced by the individual itself but also those characteristic of all the patterns (histocompatibility antigens) which it is within the capacity of the species to produce.

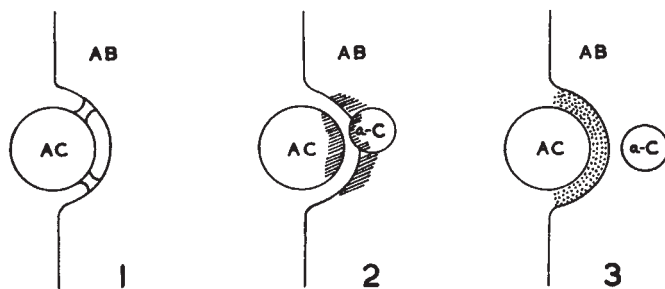


Fig. 2 A schematic diagram to indicate possible relationships of viviparous offspring (AC) to maternal tissues (AB) (see text).

The requirements can be discussed in relation to Fig. 2, which is a simple diagram of an offspring in a situation in which it is potentially parasitic on the mother's tissues. On a simple extension of the *Botryllus* situation, the cells should tolerate each other and the parasitic relationship should develop easily. In the situation of incipient parasitism that we have pictured, the biological requirement, if the parasite is to be expelled, is that the parent AB should produce a specialized cell with what in *Botryllus* would be a - type C receptor analogous to an immune receptor (cell-fixed antibody) in a vertebrate. Such a cell must be able to reach the region of contact between AB and AC and, following recognition of C, provoke a destructive local reaction. The parasitic embryonic form and the adjacent host cells will be killed, thrown off and the region repaired. The result will be wholly equivalent to the repair of a traumatic wound or of a patch of localized bacterial or other type of parasitic intrusion on a surface. Every aspect of such a reaction except what was due to the specialized anti-C cell is within the capacity of any invertebrate. Survival of any metazoan requires that it can deal with incipient infection by bacteria and repair minor injuries. Highly complex activities may be required but they do not require an adaptive immune system. To complete the sequence shown in Fig. 2 we need only to add an immunological barrier between the two sets of tissue, something with the same function as is usually ascribed to the trophoblast in placental mammals.

So far I have been considering the danger that a viviparously produced embryo in the wrong place might become a damaging parasite. Essentially the same considerations apply if we take the broader possibility of a primitive cyclostome becoming a parasite of any other available species of cyclostomes that were

not able to develop a means of protection. On this view the first evolutionary task of the free-swimming progenitors of the vertebrates was to devise a way of recognizing whether a group of living cells within its body was self or not-self. If "not-self", then in some way it must be eliminated. In retrospect one must assume that there must have developed "markers"—what we now call histocompatibility antigens by which cells genetically proper to the individual can be distinguished from foreign ones even if these are essentially similar in other respects.

Evolution of the Vertebrate Pattern

The requirement for segregation of the communities of colonial forms has sufficient similarity to suggest that the basic recognition mechanism was adapted to this new "immunological" requirement. But in the tunicates the recognition factors conduced to stable relationships between cells. As long as our potential parasite has one such "marker" in common it could, on the basis of pre-vertebrate analogies, expect to be tolerated. There are plenty of analogies, both in the history of evolution and of human war, where an initial advantage to one contestant can eventually provide the means by which it is defeated. Again thinking retrospectively, the essential change that must have been made was to produce specialized wandering cells or immunocyte prototypes in which +/− contact led not to stabilization of adjacent cell surfaces but to a potentially necrotic interaction. This would have the automatic capacity to enforce "suicide" of any immunocytes whose immune receptors (−) corresponded to (+) groupings on accessible cell surfaces and therefore ensure that only not-self (−) groupings were represented on the surviving immunocytes. It requires only a minor extension of the intra-genomic mechanism discussed in regard to *Botryllus* to ensure that (−) receptors corresponding to all the (+) receptors likely to be produced as a result of somatic (or germinal) mutation within the species should be those most commonly produced. In other words, the dominant (−) (that is, immune pattern) receptors in the primitive immunocyte population correspond to the other (+) receptors (histocompatibility antigens) that are characteristic of the species. Once again this brings us very close to the Jerne concept. At this speculative level of discussion it is immaterial whether the new patterns arise by somatic mutation or by germ-line mutation. Since there would be advantages to be gained if on the basis of what was transmitted in the germ line it was easy to ensure that all the "not-self" histocompatibility patterns of the species should be represented in the complementary patterns (−) of the immunocytes, there would be a drive to establish the necessary information in the germ line. Perhaps it is not altogether naive to suggest that when the genetic mechanism which generates diversity of immune pattern is properly understood it will be relatively easy to rewrite this concept of the evolutionary origin of immune specificity in the relevant terms. For the present, nothing would be gained by going beyond the present very general statement.

If this general approach is to serve as a stimulus to further comparative study of the evolution of immune responses, it is important to make one final point. In the lamprey and in primitive fishes there are active immune responses to homografts and delayed necrotic response to Freund's complete adjuvant, but antibody production is poor. In current immunological terminology T-responses are much better developed than B, and in line with many other indications this points to the T system—concerned with homograft immunity, delayed hypersensitivity and so on—being the more primitive. In any investigation of invertebrates or pre-vertebrate chordates for evolutionary precursors of an adaptive immune system, conventional tests for antibody production are likely to prove futile. The best approaches are probably (1) to test for differences between the responses to auto and homografts of skin or other tissue, and (2) to compare the histological response where a region has been damaged by physical means with what results from injection of material likely to induce a response

with an immunological component, such as that seen in the lamprey given Freund's complete adjuvant.

More Research Needed

The capacity to recognize the difference between self and not-self appears early in animal evolution and is well marked in at least two colonial marine forms: *Eunicella*, an anthozoan (Coelenterata), and *Botryllus*, a colonial ascidian (Tunicata). In the ascidian, Oka's work has allowed analysis of the genetics of recognition in terms not wholly remote from the histocompatibility antigens of mammalian immunology. If the current opinion is correct that vertebrates arose from the chordate larvae of some primitive intermediate between echinoderm and tunicate, it is legitimate to look at what is necessary to derive primitive vertebrate immune responses as seen in the extant cyclostomes from the recognition processes in *Botryllus*. A far-reaching reorganization is clearly necessary and the "invention" of the immunocyte as a mobile cell specialized to carry receptors for recognition of foreign pattern must have been the important step. The borderline between viviparity and parasitism may have been important as well as the very early differentiation of some cyclostomes to a semi-parasitic way of life. Without much more factual information it is impossible even to sketch the early stages. Intuitively, however, one can feel that much more extensive study of invertebrates and protochordates, particularly at the genetic level, may be specially enlightening in regard to the nature of the processes by which diversity of immune pattern arises and the evident importance of histocompatibility genes in determining the nature of that diversity, as Benacerraf, Jerne, McDevitt and others have emphasized.

"Self-recognition" in Flowering Plants

As Oka has pointed out, there are important similarities in the self-sterility of *Botryllus* colonies and self-incompatibility in flowering plants. Under the guidance of Professor J. S. Turner and Miss Mary Ellis, I have consulted enough of the recent literature on self-incompatibility in plants to feel that it might be helpful for an immunologist to offer some comments on self-incompatibility in plants in the light of the foregoing discussion of the evolution of self-recognition in animals.

Self-incompatibility is very common in flowering plants and has been well known since Darwin's time. There is much to suggest that by enforcing heterozygosity it has been of great importance in the "explosive success" of angiosperm evolution. There are a number of different mechanisms, but the commonest and most widely studied example is that shown by failure of the haploid pollen tube to grow through the diploid stilar substance and to effect fertilization²⁰. Many but not all such examples are controlled by a single locus with a very large number of possible alleles. In order to keep the topic as simple as possible I shall confine myself wholly to this class. It has been studied in *Petunia*, *Lilium*, *Oenothera*, *Trifolium* and *Nicotiana* among others.

In general the phenomenon of self-incompatibility takes the following form. Pollen from an individual plant is incapable of setting seed in flowers of that plant or in other plants of the same incompatibility group, that is, with a common, S¹ say, self-incompatibility allele. S¹ pollen, however, is fully active in fertilizing any other plant of the species. Similar findings hold for other types of pollen. The number of distinct S alleles in a species can be very high: seventy-eight are mentioned in red clover and more than twenty-four in cherries. The condition is usually symmetrical, but in some species the incompatibility is one-sided in the sense that pollen from plants of strain A will fail to fertilize strain B, but B pollen can fertilize strain A.

To take a classical example. Crane and Lawrence²¹ tabulated fifty-two varieties of cherry to show that they fell into twenty-four compatibility groups, the three largest containing

nine, eight and four varieties respectively. All the incompatibilities shown in the table are symmetrical. This is, however, by no means universal among cultivated fruits; there are some strikingly one-sided examples among plums, and apple varieties in general show quantitative differences in the capacity to set seed instead of the relatively clear cut results + or - of cherries. Confining ourselves to the latter, the failure of the pollen tube to develop in styles of self type must be looked on as a positive recognition mediated by components in pollen tube surface and stilar tissue, both of which are coded for by a common allele. The ability for the pollen tube to grow and reach the ovule in any other strain is at this level a negative finding.

In a recent discussion of the general problem, Lundqvist²² has described three essential genetic features. First, the S allele has two sites (pollen tube and stilar tissue) for phenotypic expression. Second, these two gene products are exactly coordinated to bring about incompatibility by their interaction. Third, a changed allele may lose its characteristic phenotypic expression at one site while fully active at the other.

If only the usual symmetrical form of self-incompatibility is considered, plants seem formally similar to *Botryllus*, as Oka had pointed out. If instead of using S¹, S² and so on for the alleles we use the A, B, C and D nomenclature, as for the colonial ascidians, the rules governing the behaviour of heterozygote forms are as follows for two plants AB and CD. Both are self-incompatible, that is, when either A or B pollen grains lodge on the stigma of an AB flower, growth of the pollen tube is inhibited. When A or B pollen lodges on CD, however, the combinations are all fertile and AC, AD, BC and BD offspring can be obtained. When these are mated, the types of offspring are:

♀ AD × AC ♂	AC, CD	♀ AC × AD ♂	AD, CD
BC × AC	AB, AC	AC × BC	AB, BC
BD × AC	AB, AD, BC, CD		

These are essentially the same rules as obtain in *Botryllus*. The symmetry of the reaction is not always complete and it is well known that irradiation of pollen will often allow fertilization of the normally self-incompatible strain from which it was obtained. This has made it possible to produce homozygous plants which still cannot be fertilized by normal pollen of the original strain. There are also a number of natural examples of one-sided incompatibility as well as various degrees of partial compatibility. One gains the impression that as in so many other genetic phenomena an intensely complex set of genetic factors is concerned and that specially suitable material must be chosen to provide a system expressible in simple terms. The change from self-incompatibility to compatibility is operationally extremely easy to detect and measure and it has therefore attracted many workers. Perhaps one of the most striking of their results is that irradiation is more likely to delete an S allele than to produce any other type of mutant.

At the physiological and biochemical level there is much on record about specific situations, but few generalizations have been established from the several examples of self-incompatibility in different species. A number of writers, notably Linskens²³, have spoken of antigen-antibody reactions and applied serological methods. Lewis²⁴ identified individual antigens in pollen extracts of *Oenothera* by Ouchterlony methods and correlated each with a specific incompatibility allelotype. He obtained similar findings in the stilar tissue of *Petunia*, while Linskens, using radioisotopic labelling, was able to show that a complex of pollen protein and style protein was formed in the course of incompatible pollination. Knox *et al.*²⁵, using immunofluorescence techniques, showed that most of the antigens capable of provoking antibody in rabbits immunized with simple saline extracts of ragweed pollen were located in the inner (intine) part of the cell wall of the pollen grain. Studies by immuno-electrophoresis showed that their pollen extracts contained at least seven antigens, some of which were

likely to be enzymes known to be present in the intine. They noted, however, the possibility that some of the wall-associated antigenic material could be concerned with incompatibility reactions. One might add that it would be even more interesting to look for a possible relationship in some appropriate species between the incompatibility protein coded by the S allele and the protein responsible for allergic reactions to the pollen in human subjects.

The nature of the process by which the pollen tube fails to grow effectively is of no obvious importance in the present context. It is said that there are various morphological changes—the generative nucleus fails to divide and the vegetative nucleus disappears—and, as would be expected, there are a variety of enzymatic changes. From our point of view, the nature of the specific interaction between a pollen substance and a style substance is the important matter. What happens after that has no bearing on vertebrate immunology.

There are many reasons for avoiding the use of the terms antigen and antibody, but we can legitimately use the concept of mutual recognition between cell surfaces. The facts of self-incompatibility in plants demand that there is a positive recognition by which a pollen tube carrying allele A has a surface protein which on "recognizing" another protein in the style also carrying allele A interacts with it, so triggering the progressive degeneration of the pollen tube. Strictly speaking, some substance other than protein might be concerned, but from every point of view the concept that the substance is a protein directly coded for by the allele seems to be the only one worth consideration. All the current hypotheses make this assumption.

Probably only two basic hypotheses are possible. Both agree that one allele codes for proteins in two different sites. Both have been formulated by Lewis, the second being the one he currently favours. In the first, the allele codes for two distinct proteins, one for the pollen tube, one for the style. The proteins S^p and S^s have a complementary configuration analogous to antigen-antibody or enzyme-substrate. In the second, the allele is a complex which codes for (a) specific protein pattern common to both sites; (b) activator for protein production in pollen tube, and (c) activator for protein production in style. For reasons associated with the fact that grasses have two loci, S and Z, concerned with self-incompatibility, Lewis postulates that the primary gene product takes the form of a dimer. When this makes contact with an identical dimer on the interacting surface, union, presumably involving an allosteric molecule, occurs and the tetramer functions as a growth inhibitor. The idea is admittedly based on the structure of haemoglobin or immunoglobulin G, and, as Lewis²⁶ himself points out, cannot even be tested experimentally until the dimer and tetramer molecules are available for physical and chemical evaluation.

No one seems to have claimed that a decision can yet be made between the two possibilities: interaction of two identical proteins (or polypeptide determinants) or of sterically complementary patterns.

Simply because I am primarily concerned only in looking for analogies with immunological processes that involve the recognition of self from not-self, I should like to elaborate Lewis's first hypothesis of sterically complementary proteins along the lines we have adopted for *Botryllus*. The concept then becomes for S alleles A and B that as the pollen grain germinates and the tube begins to grow, a specific interaction between chemical groupings A + on pollen tube, A – in the cells of the style and/or A – on pollen tube, A + in the cells of the style results in cessation of growth. In view of the symmetry of the relationship, we assume that both + and – patterns are present on both sides and that the cells of style, being diploid, will carry + and – patterns of each gene A + B.

Because there are many plants which are self-fertilizing and more which show minor degrees of self-incompatibility, it is probable that + – contact is merely an initiating signal which, depending on other evolved cellular mechanisms, may be

neutral, stimulatory or inhibitory as in self-incompatible species and varieties. Without having any detailed knowledge of the process of morphogenesis in plants, one can postulate that, as in animals, there must be some interchange of information between adjacent cells, part at least of which will take the form of + – union between specific patterns. The point to be made is simply that the possibility and the need for inter-cellular information exchange has provided an opportunity for the evolution of means of recognizing the difference between self and not-self when this was required for evolutionary purposes, whether to prevent self-fertilization or to produce an adaptive immune system.

The still totally unresolved problem of how a single allele can produce two mutually reactive proteins in the two relevant sites again draws attention to the currently teasing question in animal immunology as to how the capacity to produce a certain type of antibody is firmly linked to a specific histocompatibility antigen. Obviously the answer must come from the isolation of the reactive S proteins in pollen tube and stylar tissue, and assuming that they are polypeptides to determine the difference in amino-acid sequence corresponding to allelic change.

Reverting once more to Jerne's¹⁶ concept that the organism "knows" what are the structures of the histocompatibility antigens which the species can produce, we can hardly avoid pondering—heretically, no doubt—whether there is any genetic way by which a nucleotide sequence coding for an amino-acid group A–B–C–D can automatically produce another sequence P–Q–R–S (say) which can "recognize" it. One has an intuitive feeling that the "choice" of the twenty biological amino-acids and the form of the genetic code might in the last analysis depend on some such requirement for mutual recognition.

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Quantitative Determination of Mononucleotide Conformations in Solution using Lanthanide Ion Shift and Broadening NMR Probes

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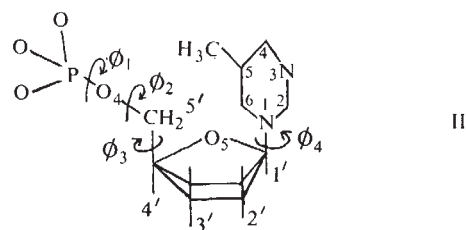
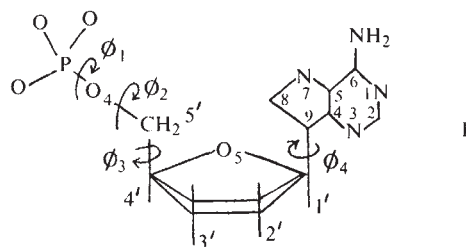
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A computer treatment is developed for determining the conformations of flexible molecules, such as nucleotides, in aqueous solution.

EFFORTS to determine quantitatively the conformations of flexible molecules (including nucleotides) in aqueous solutions—involving high resolution nuclear magnetic resonance (NMR)¹⁻³ and spin-echo studies using Mn(II) as a probe⁴—have been difficult because of the complexity of the chemical shift phenomenon and the interpretation of correlation times.

We wish to show how these problems can be avoided by a general procedure. We use the determination of the selective chemical shifts and broadenings of the proton NMR signals of compounds in aqueous solutions containing added paramagnetic ions of the lanthanide series. A computer program (incorporating calculation and display facilities) analyses the raw data by searching for conformations that are possible sterically, and which agree with the NMR data. This report gives the general approach and the results for two representative mononucleotides, 9-β-D ribofuranosyladenine-5'-monophosphate (AMP), I, and 1-β-D deoxyribofuranosylthymine-5'-monophosphate (TMP), II. A later report will deal with dinucleotide conformations; wider applications using different metal probes, different media and larger molecules will be reported on the basis of work in progress. A conformation is here defined as the ensemble average seen by our physical techniques.



The lanthanide cations, when attached to organic molecules, have previously been observed to be useful probes for selectively perturbing the normal NMR spectrum. This perturbing effect has so far been used in an effort to reduce the complexity of second order NMR spectra^{5,6} and to calculate the distances of analogue substrates from the active sites of enzymes using both NMR and ESR spectroscopy⁷. Such applications do not reveal the full potential of the lanthanide cations as NMR probes, which can only be appreciated by observing that these cations,

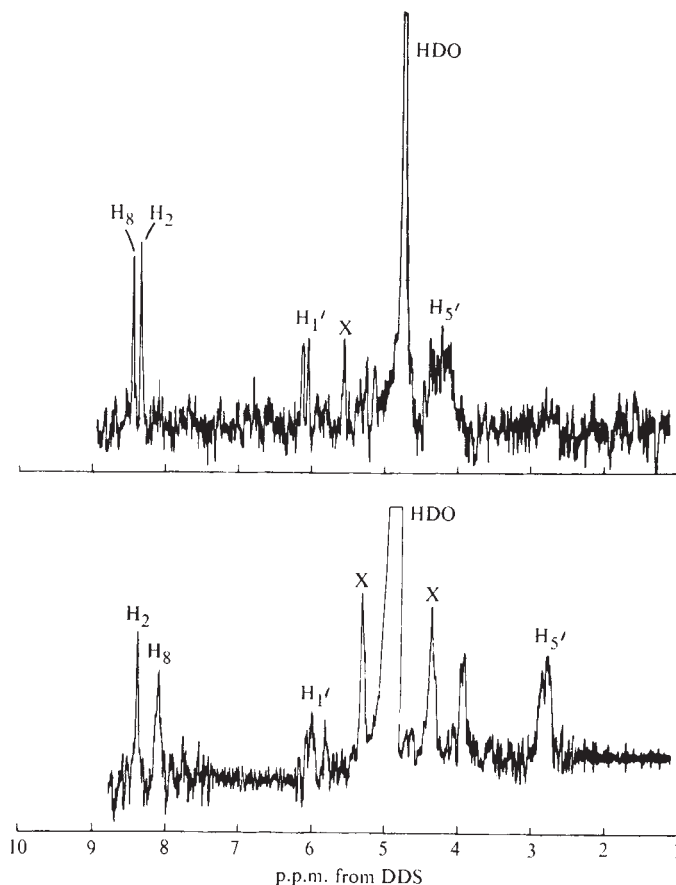


Fig. 1 Spectra of 0.028 M AMP (upper) and 0.028 M AMP with addition of 0.28 M Eu(III), pH 2.0. Both spectra are referenced to DSS as an internal standard.

Rare earth solutions were made by dissolving their purified oxides (in the case of europium and holmium) or nitrate (in the case of gadolinium), in DCl and adjusting the nominal pH to approximately 2. Shift measurements were made in the range of metal ion concentrations of 0.0 to 0.5 M. The nucleotides studied in this work were obtained from Sigma, London, as 'Sigma Grade' purity. Both AMP and TMP were titrated to the proper pH from solutions of their sodium salts. Solutions were made with concentrations of nucleotide of the order of 0.025 M from materials twice lyophilized from D₂O. NMR spectra were obtained with the use of a JEOLCO C60-H high resolution spectrometer. The probe temperature was $28^\circ \pm 1^\circ$ C. Standard 5 mm sample tubes were used.

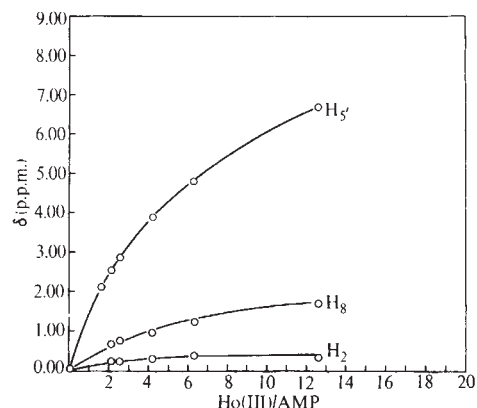


Fig. 2 Typical titration curve of observed chemical shifts against metal ion : nucleotide ratio for various protons on AMP after addition of Ho(III).

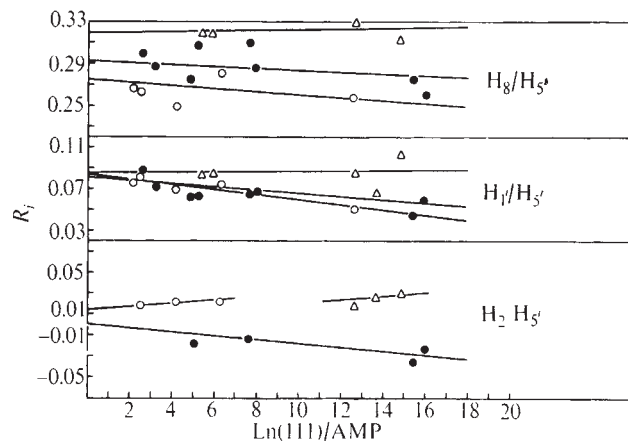


Fig. 3 Shift ratios obtained from raw data for various protons on AMP as a function of metal ion : nucleotide ratio for various metal ions. ●, Eu(III); △, Nd(III); ○, Ho(III).

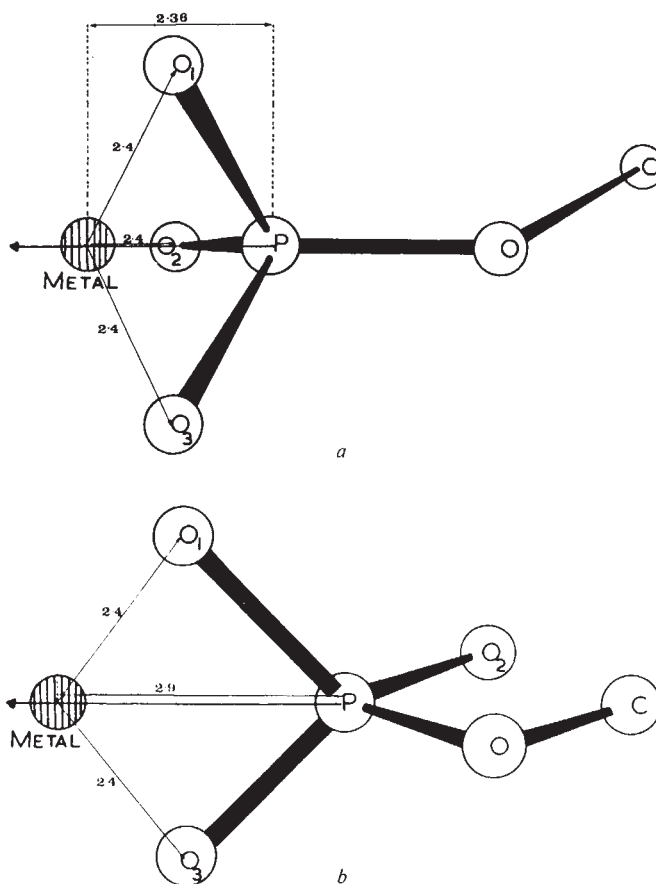


Fig. 4 *a*, Initial scheme for site of metal ion with respect to phosphate group. No solutions were found for this scheme with the symmetry axis of the metal ion pointing along the central phosphorus oxygen bond. *b*, Final scheme for site of metal ion with respect to phosphate group; applies to both AMP and TMP.

possessing large paramagnetic moments, divide themselves into three classes, as follows:

(i) Those such as Eu(III) and Nd(III) which have very short electron relaxation times. They have large interactions with nuclei which cause chemical shift perturbations but negligible line broadening.

(ii) Those such as Gd(III) and Eu(II), that is $4f^7$ cations

properties and are expected to bind to any organic molecule in a very similar structural and chemical manner. Moreover, because the $4f$ electron shell is well screened, there is little overlap between the unpaired electron density on the lanthanide and the ligand electrons. We therefore feel able to neglect contact effects and assume that all perturbations of proton resonances are attributable to pseudocontact terms (as long as

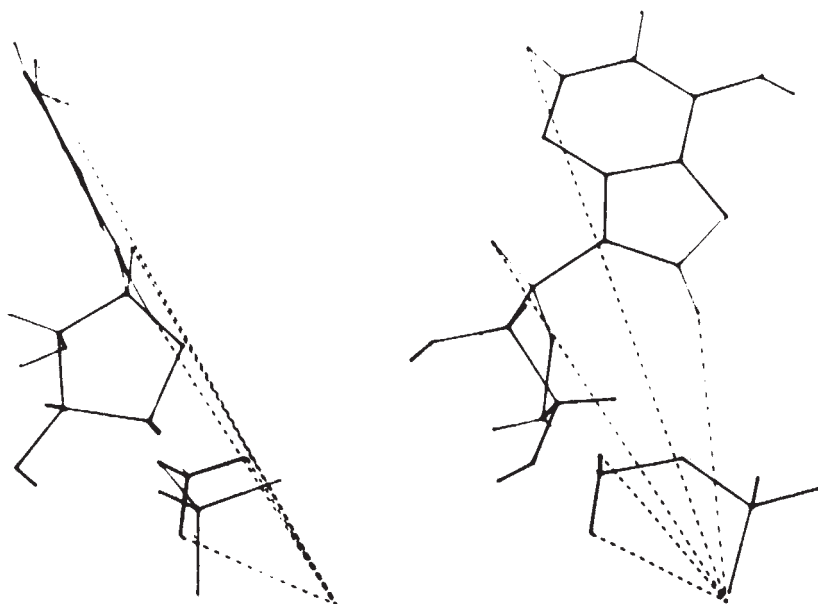
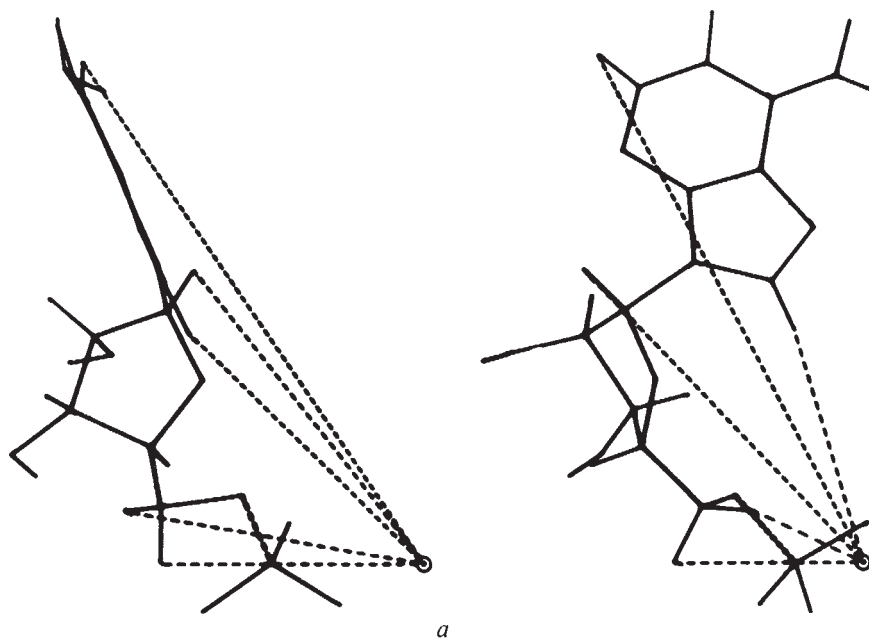


Fig. 5 Crystal (upper) and average solution (lower) conformations of AMP. *a*, Orthogonal views; *b*, stereo views.



a

(ref. 7), that have long electron relaxation times and singlet ground states. They cause large isotropic broadening effects.

(iii) A group of cations (such as Ho(III)) with intermediate relaxation times which cause a considerable degree of anisotropic broadening as well as chemical shift perturbations.

Unlike the available transition metal cations in the series Mn(II) to Cu(II), lanthanide cations have very similar chemical

the perturbed nucleus is not in the immediate coordination sphere of the metal). Fortunately, as we will show, this assumption can be tested experimentally and so, in contrast to shifts caused by other paramagnetic ions^{8,9}, no empirical separation of contact¹⁰ and pseudocontact shifts is necessary. We can now use the shift equation governed by anisotropic pseudocontact interaction¹¹

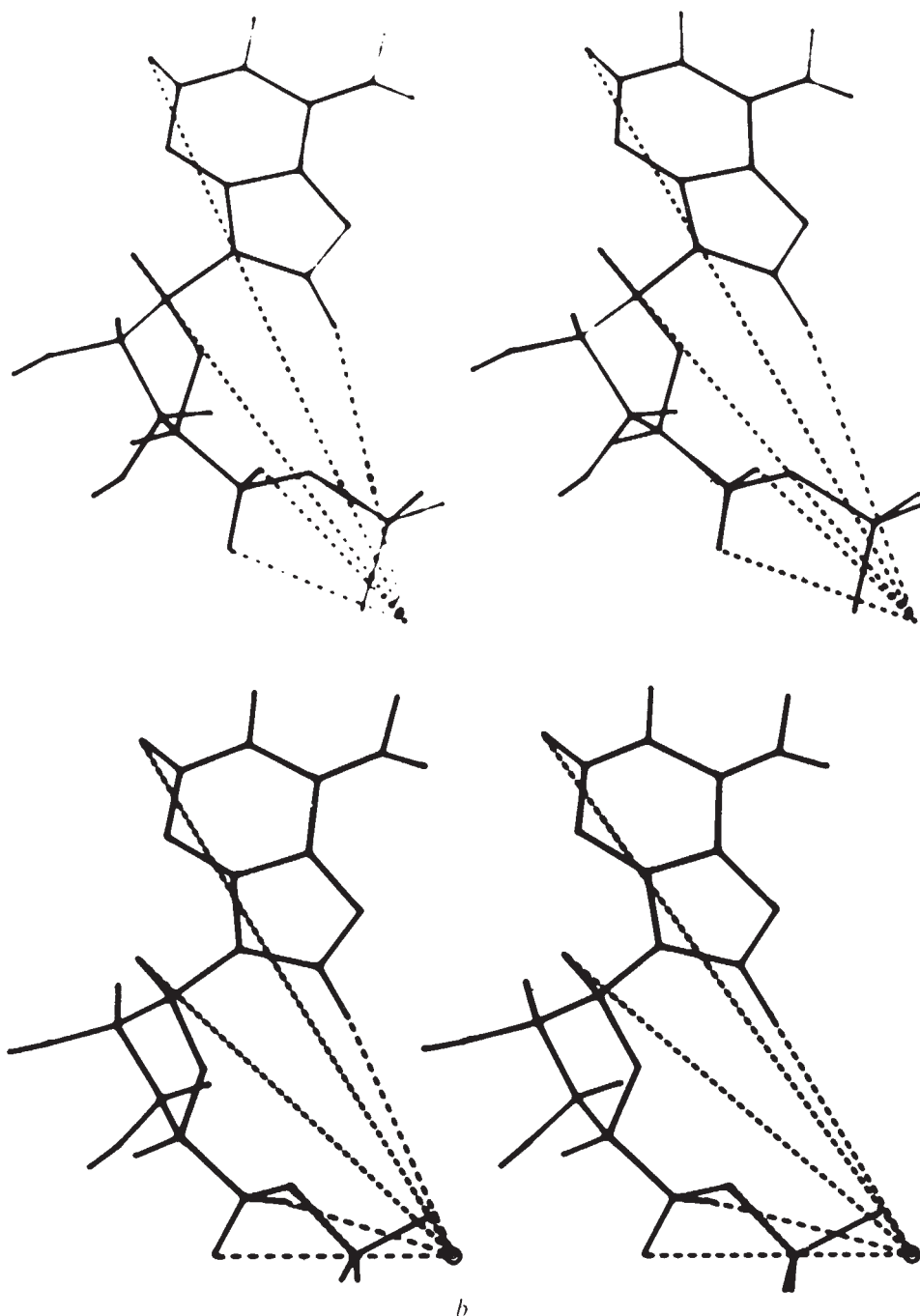
$$\frac{\Delta \nu_i}{\nu_0} = D \left\langle \frac{(3\cos^2\chi_i - 1)}{r_i^3} \right\rangle \quad (1)$$

where $\vec{r}_i$ is the distance from the metal to the i th proton on the ligand and χ_i is the angle between the vector, $\vec{r}_i$, $\vec{r}_i$ and the prin-

In the case of ions of class (ii), a general equation governing the half-height width of the perturbed signals is^{7,12}

$$\delta \nu_{i,1} \propto 1/T_{2,i} = F(\gamma_i, \mu_{\text{eff}}, \tau)/r_i^6 \quad (2)$$

where μ_{eff} is the effective paramagnetic moment of the metal ion, γ_i is the nuclear magnetic moment and τ is the correlation time for magnetic perturbations.



cipal symmetry axis of the complexed paramagnetic ion, averaged over motions rapid on the NMR time scale. D , a function of temperature and the magnetic properties of the complex, is a constant for a given metal independent of the nuclear resonance under inspection. It is clear from this equation that measurements of shifts in complexes containing lanthanides yield angular and distance information.

The third group of cations can be used in two ways. First, shifts may be measured and the data used to prove the origin of shifts as a result of pseudocontact terms. Second, the anisotropic broadening may be measured and by the use of standard expressions for the broadening¹³, including the magnetic properties of the complex, the conformation deduced from the use of classes (i) and (ii) probes confirmed.

From these equations we can determine quantitative internal angles and distances for molecules which interact with lanthanide ions. We now show how we have derived the conformations of AMP and TMP. We have worked with the nucleotide phosphate mono-anions at a nominal pH of 2.0 and, as self-stacking effects are known to be important in aqueous nonnucleotide solutions the concentrations of which are above 0.03 M, our experiments have been restricted to concentrations at or below this value.

Fig. 1 shows the spectra of 0.03 M AMP before and after addition of excess Eu(III), a class (i) cation, in $\approx 10:1$ ratio. Assignments are based on previous high resolution NMR data for the free nucleotide¹⁴ and by considering the dependence of the shifts of the unbroadened lines on Eu(III) concentration. The protons most shifted are H₈ and the H_{5'} protons. The H₂ and H_{1'} protons are much less affected. On addition of Ho(III), the direction of the shifts is reversed in the corresponding spectra for AMP and the lines differentially broadened. In control experiments with adenosine, no shifts were obtained. Shifts were obtained for the protons of ribose monophosphate. The lanthanides were therefore assumed to bind to the phosphate.

Fig. 2 shows the titration curves for AMP obtained with a range of Ho(III) concentrations. From these curves association constants for the process,



are easily derived. The value for Ho(III) is $K_a = 6 \pm 2$ at 28° C. For Eu(III) complexes, the value for AMP is 10 ± 2 at 28° C and for TMP is 17 ± 3 at 28° C. With the available signal to noise ratio on our instrument we could not lower the nucleotide concentration enough to result in saturation of the shift effect even at the highest Ln(III) concentration. The results show that in the range of concentrations used here only a one-to-one Ln(III) : nucleotide complex is formed.

In the systems nucleotide + Ln(III) the two H_{5'} protons are nearly magnetically equivalent—as they are before perturbation. TMP is a derivative of deoxyribose and we observe the two non-equivalent H_{2'} protons. These form the AB part of an ABX multiplet. Unfortunately, when a class (i) probe is used at 60 MHz this multiplet is hidden under the H₅ peak at high Ln(III) concentrations and we are not able to measure the H_{2'} chemical shifts as precisely as the others. The observed equivalence of the H_{5'} protons in both AMP and TMP does, however, provide added information.

The results of experiments using a class (ii) isotropic selective broadening cation, Gd(III), in concentrations of the order of 10^{-4} M, show no selective shifts of the AMP lines. Broadening effects are of the order: $\text{H}_8 \approx \text{H}_5' \gg \text{H}_{1'} \approx \text{H}_2$.

Our data analysis begins with the observation that all peaks in the Eu(III) experiments are single and sharp, which leads to the conclusion that exchange of paramagnetic ion between the free and bound states is rapid (in agreement with our finding of small association constants). The control experiments with ribose phosphate and adenosine indicate that the metal cation binding site is, as expected, near the ionized phosphate.

The procedure for data analysis follows. We form ratios of the observed shifts from a standard proton, for example, the two equivalent H_{5'} protons, at each metal ion concentration used, and then extrapolate these ratios to zero metal ion concentration. The results for AMP are shown in Fig. 3. We believe that the scatter of approximately 10% in the ratios extrapolated from the raw data for the different lanthanides is a result of a bulk susceptibility effect on the chemical shifts^{15,16}. The correction for this, necessary in all paramagnetic solutions in which the shifts are related to internal references, is small and neglected here because we have no means for determining it quantitatively. In the case of the H₂ proton of AMP and the H₅ protons of TMP, the observed shifts were zero within our reading error except at high Ln(III) concentrations.

As already stated, for purely pseudocontact shifts the ratios should be independent of the nature of the metal ion. Within

the 10% factor Fig. 3 shows this to be true for Eu(III) with six 4f electrons, Ho(III) with ten 4f electrons and Nd(III) with three 4f electrons. We therefore believe that our assumptions that contact shifts are negligible and that the binding sites of chemically similar lanthanides are the same are substantiated. From equation (1) the set of extrapolated ratios are of the form

$$R_i = \frac{\Delta v_i}{\Delta v_o} = \frac{\left\langle \frac{(3\cos^2\chi_i - 1)}{r_i^3} \right\rangle}{\left\langle \frac{(3\cos^2\chi_o - 1)}{r_o^3} \right\rangle} \quad (3)$$

The set of broadening ratios from the Gd(III) experiment, which need no extrapolation because the metal ion is present in very low concentrations, is of the form

$$\frac{\delta v_{i,1/2}}{\delta v_{o,1/2}} \propto \frac{1/T_{2,i}}{1/T_{2,0}} = \frac{r_o^6}{r_i^6} \quad (4)$$

from equation (2).

Given the sets of ratios we could now have proceeded as follows. A starting geometry might be assumed for the metal : nucleotide complex, which might then be tested to see whether the derived angle-distance factors from equation (3) give the correct shift ratios, R_i , and whether the proposed distance ratios are in agreement with equation (4). If this is not the case we assume another geometry, apply the tests again, and so on. Two problems emerge. First, what limits are acceptable for the definition of a conformation? Second, is there more than one conformation consistent with the data and, if so, how do we find these conformations? There are four bonds in AMP and TMP about which intra-molecular rotation is possible and if we considered conformations differing in steps of a few degrees rotation about each of these there are clearly several million different conformations. For each model conformation, according to our trial and error procedure, angle and distance measurements are required to form the R_i ratios which must then be compared with the observed values. This clearly precludes the possibility of a manual search for the conformation using mechanical space-filling models.

Table 1 Minimum Pair Contact Distances in Å

	C	N	O	P	H
C	2.8	2.6	2.6	3.2	2.3
N	2.6	2.5	2.5	3.0	2.2
O	2.6	2.5	2.5	3.0	2.1
P	3.2	3.0	3.0	3.2	2.4
H	2.3	2.2	2.1	2.4	1.9

With high speed computers and graphical displays it has become possible to replace conventional mechanical models by the use of interactive programs^{17,18} in which a three dimensional representation of the molecular conformation is achieved by means of a suitable stereoscopic pair of diagrams on the screen of a cathode ray tube. The computer installation which we used consists of a Ferranti 'Argus 500' computer equipped with a Ferranti System 30 CRT display. The program used was originally developed in connexion with the interpretation of electron density maps derived from X-ray diffraction studies (C. D. B. and A. C. T. N., to be published) and a routine to calculate the NMR shift ratios for each conformation considered by the program and to display their

values simultaneously with the molecular diagrams has been added. The program also takes into account the space-filling nature of the atoms and conformations may be rejected in which atoms are closer together than pre-specified distances. The program considers the molecule as a set of rigid groups of constant geometry linked by single bonds about which rotation is possible. Program input consists of a set of coordinates consistent with the molecular stereochemistry, such as can be obtained from single crystal X-ray studies, a set of van der Waals radii for the various atomic species and information on the single bonds about which rotation is possible.

many interatomic distances are not affected by all the single bond rotations. For example, the van der Waals contact between H_5' and O_5 depends only on the value of ϕ_3 . Thus, only trial structures having ϕ_3 within a permissible range go forward for subsequent examination by the NMR shift filter. By choosing a suitable order for testing the various rotations it is possible at each stage to reduce the number of conformations to be examined.

Such filters must, of course, be used with care and with due regard for the precision of the experimental parameters involved. Thus, it is necessary to use "outer limit" (that is,

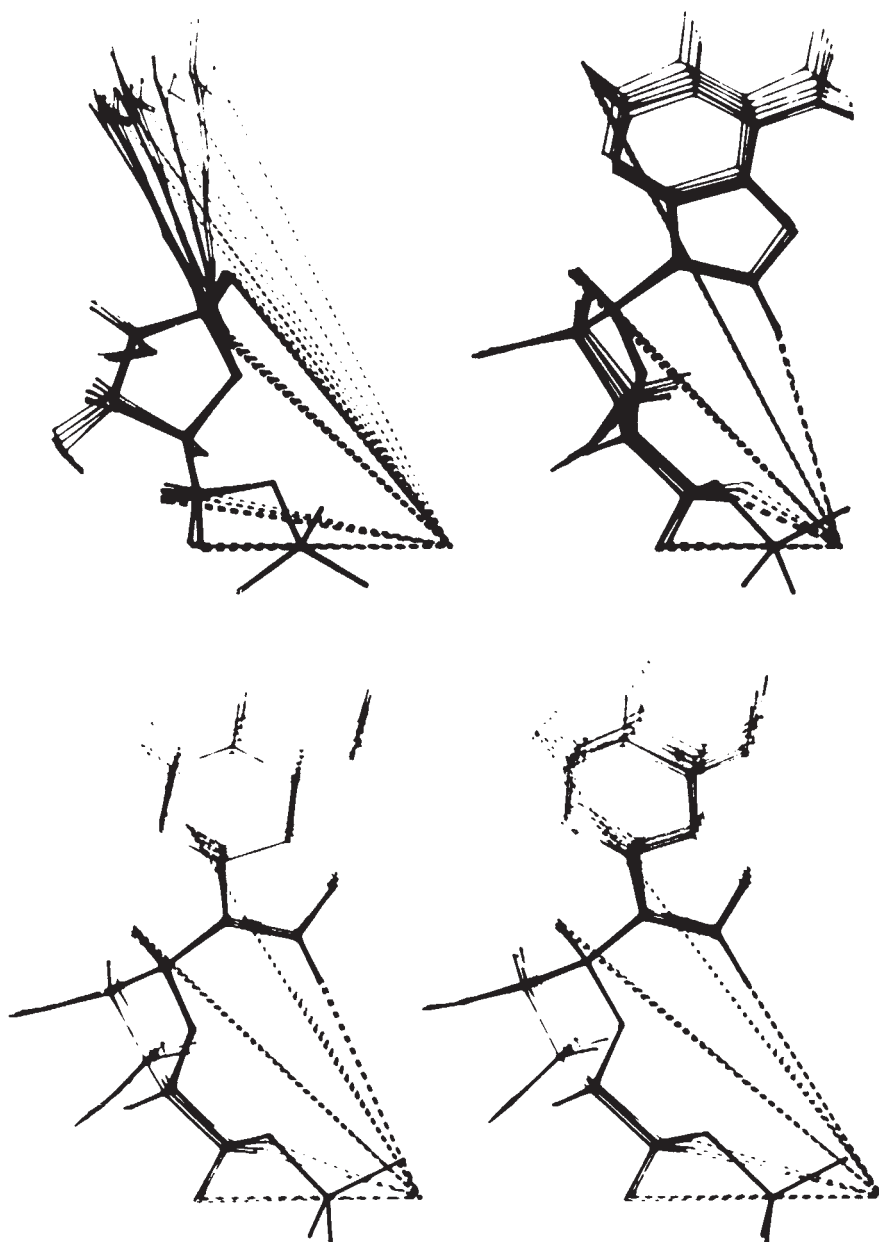


Fig. 6 Family of solution conformations of AMP, orthogonal (top) and stereo views.

This computer treatment permits molecular conformations to be scanned much more rapidly than with mechanical models and permits a direct output of the parameters of promising conformations. As described, however, it does nothing to reduce the total number of conformations to be examined. Nevertheless, a very substantial reduction can be achieved by using the constraints imposed by the van der Waals contacts and NMR shifts as "active filters" rather than by using a simple trial and error procedure, the basis of which is the fact that

minimum observed) van der Waals distances (Table 1) rather than normal contact distances in order not to reject any remotely possible conformation. In practice, this filter only reduced the total number of conformations to be tested by a factor of two. The precision of the NMR results was considered to be such that only those ratios within the experimental scatter, such as shown in Fig. 3, were accepted. The NMR ratios proved to be very effective filters, as shown in Table 2, leaving us with only a small number of solutions.

A further feature of the program permits the translation of the molecule along a coordinate axis by a fixed amount after each complete cycle of the angles. Because the metal ion is assumed to be placed at the origin of the coordinate system describing the molecule, this allows various metal coordination schemes to be examined.

The computer techniques outlined have been applied to the NMR data collected for AMP and TMP. Initial coordinates for the program were obtained from single crystal X-ray studies^{19,20} and an angle scan of 4° was chosen empirically to be compatible with the tolerance set on the NMR filter. Two

described previously but with the metal ion in the same position. This is chemically highly improbable and therefore we tried a second scheme (Fig. 4b) in which the orientation of the symmetry axis with respect to the ester axis is 70° but the metal ion is now located in the plane containing the phosphorus atom and only two oxygen atoms, O_1 and O_3 along an axis bisecting the O_1-P-O_3 angle. The symmetry axis is now along this latter axis, which is chemically a logical situation because we are considering the mono-anion form of the phosphate group. With this scheme, solutions were obtained for both AMP and TMP. For Eu(III), as an example, metal ion-

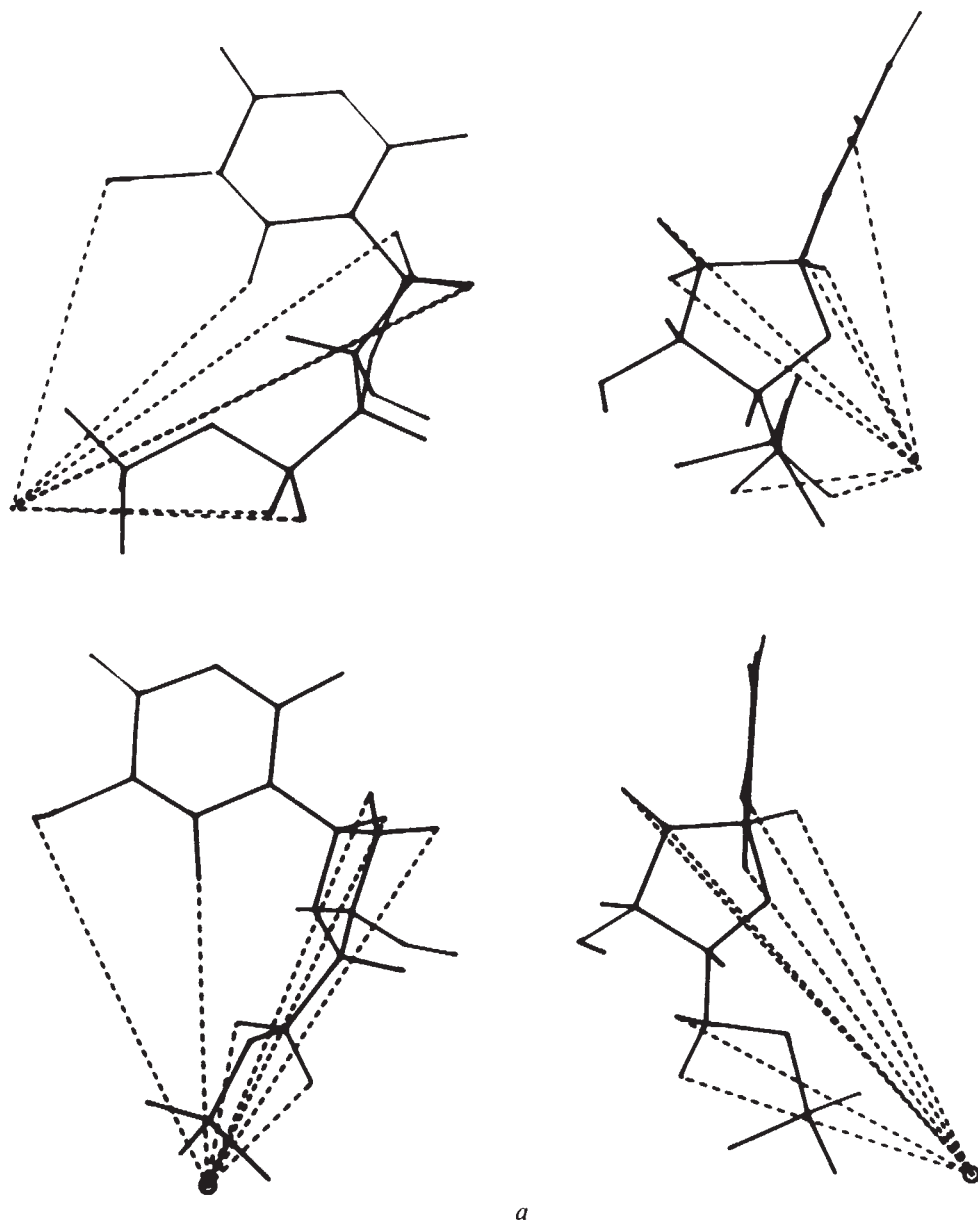


Fig. 7 Crystal (upper) and average solution (lower) conformations of TMP. *a*, Orthogonal views; *b*, stereo views.

different coordination schemes have been examined for the phosphate metal complex, in both of which the metal symmetry axis was directed towards the phosphorus atom. Fig. 4a shows the first scheme tried, which involved equal association of the lanthanide with all three phosphate oxygen atoms, the metal lying along the phosphate ester bond axis. No satisfactory solutions could be found because neither the angle nor the distance factor in the expression for the ratio of the H_1 to H_3 shifts could account for this ratio being as low as 0.08. Formally acceptable solutions were, however, obtained with the metal ion symmetry axis directed at an angle of 70° to the axis

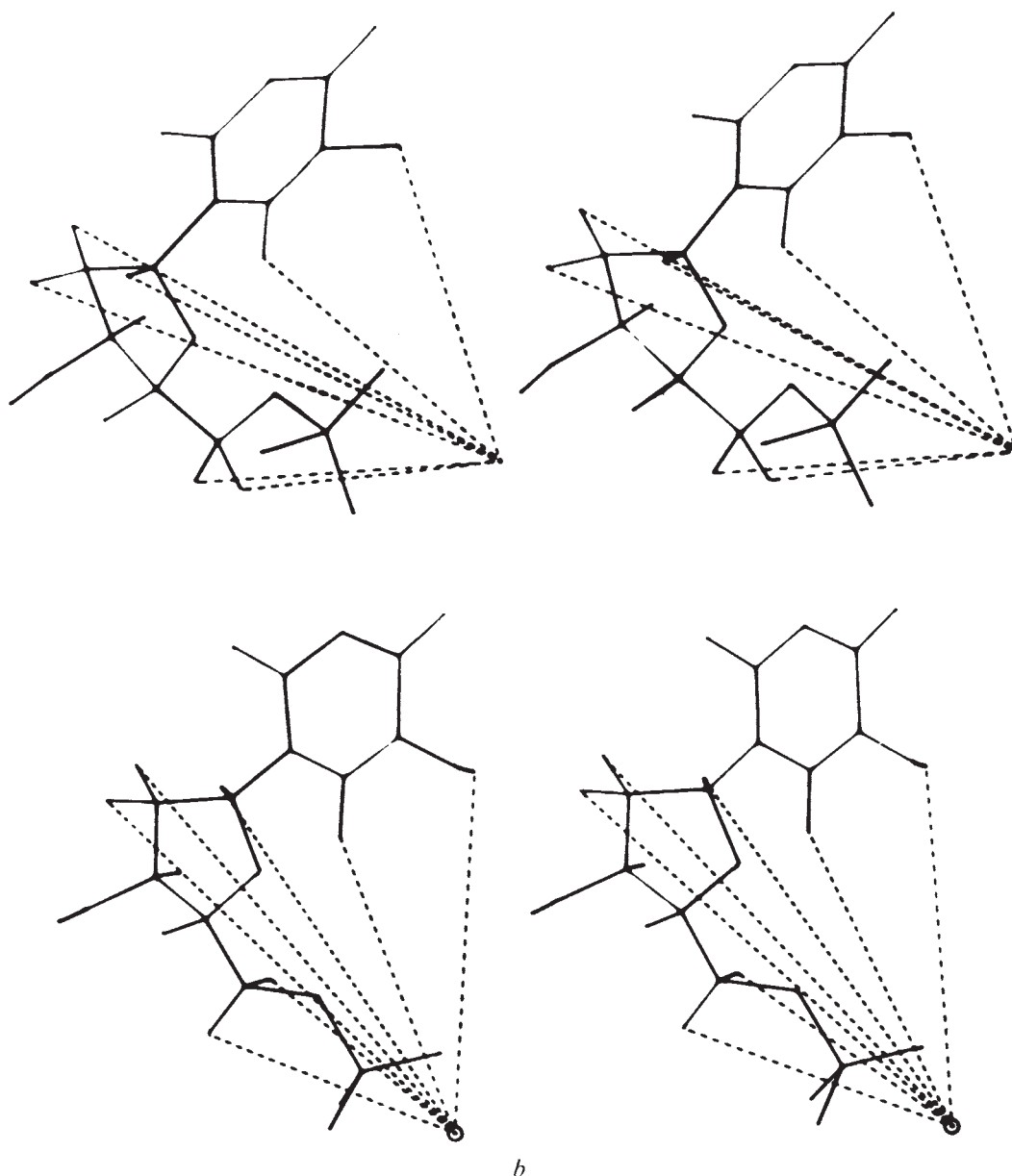
phosphorus distances of up to $4.3 \text{ \AA}$ in the case of AMP and $3.5 \text{ \AA}$ for TMP gave solutions. As the assumed minimum contact distance for O-Eu(III) is $2.4 \text{ \AA}$, this effectively places a lower limit of $2.9 \text{ \AA}$ on the P-Eu(III) distance although the distance for a hydrated Eu(III) ion lies outside the range in which possible conformations are found. Thus we conclude that the Eu(III) ion in the complex is not a second sphere complex and is $2.9 \text{ \AA}$ from the phosphorus.

Examination of the 195 conformations produced by the computer for AMP reveals several geometrically distinct families. Only twelve members, all from one of these families,

had distance factors that were consistent with Gd(III) line broadening data (Figs. 5 and 6). In the case of TMP there were forty-eight possible conformations of which four, all of one family, were selected by broadening data. The fact that families of conformationally similar solutions are found, rather than a unique conformation, is a consequence first of the tolerance placed on the fitting of the NMR ratios and second that a range of rotational angles produce much the same spatial arrangement of the nuclei observed in the experiment. The availability of further data for the H_2' protons in TMP accounts for the smaller spread in angles than for AMP.

portion alone (three degrees of freedom and five observed protons).

Tables 3, 4, and 5 summarize the coordinates of the most satisfactory average conformations. Figs. 5-8 illustrate various aspects of the final results. The X-ray work on AMP was evidently performed on the singly ionized phosphate form¹⁹. The counter-ion in this work was not reported. The TMP work²⁰ was done with the doubly ionized phosphate form, with Ca(II) as the counter ion. Our studies show that the orientations of the bases with respect to the sugar ring for solvated AMP and TMP are very similar to their corresponding



Extension of these studies to larger molecules with more internal degrees of freedom will increase the computer time necessary for interpretation of data. But, provided the observed protons are distributed throughout the structure, the filtering technique will keep the number of conformations to be examined from increasing as rapidly as the total number of possible conformations. The effect of the filters will vary with the molecule studied, but their effectiveness may be gauged from the fact that the number of conformations examined in the 4° scan of TMP (four degrees of freedom, seven observed protons) is no more than that examined for the sugar phosphate

orientations in the crystals. There is some movement of the phosphates with respect to the crystalline case in the solvated species. We found, in accord with previous studies^{1,2}, that the conformations are essentially *anti*.

Similar experiments with other mononucleotides have been performed and in all cases selective shift and broadening effects have been observed. The studies have been extended to dinucleotides with equal success, and a study of their conformations and structural transitions will be the subject of a succeeding communication. We also have evidence that the method can be used to study the conformations of many other types of

molecules. It should be emphasized that the method can be extended to several other nuclei. In particular, ^{13}C spectral measurements of pseudocontact shifts and broadening effects, in combination with proton measurements, will enable quite precise conformational determination in solution for many molecules.

In conclusion, a method has been developed for the quantitative study of molecular conformations in solutions, which

Table 2 Calculation Statistics for Two Mononucleotides

	AMP	TMP
Number of protons observed	5	7
Number of bonds rotated = N	4	4
Angle increment in degrees = A	4	4
Total number of conformations $(360/A)^N$	64×10^6	
Estimated number of conformations without bad contacts	20×10^6	
Number of conformations scanned after ϕ_3 filter	32×10^3	14×10^3
Number of conformations consistent with both NMR shift and van der Waals radii	195	48
Number of conformations consistent with broadening ratios	12	4
Computing time with Argus 500 (add time = 6 μs)	2 h	1 h

Table 3 Extrapolated Shift and Broadening Ratios, Averaged and Normalized to H_5' , for the Systems $\text{Ln(III)} : \text{AMP}$ and $\text{Ln(III)} : \text{TMP}$ and Tolerances Within Which Computer Search was Performed

		AMP			
Shift	Ratio	H_8/H_5'	H_2/H_5'	H_1'/H_5'	
	Tolerance	0.29	0.00	0.08	
		0.32 to 0.27	+0.02 to -0.02	0.10 to 0.07	
Broadening	Ratio	1.0	—	—	
	Tolerance	± 0.5	—	—	
		TMP			
Shift	Ratio	H_6/H_5'	$\text{H}_3/\text{H}_5'^*$	H_1'/H_5'	H_2'/H_5'
	Tolerance	0.39	0.00	0.07	0.20
		0.41 to 0.38	+0.15 to -0.15	0.09 to 0.06	0.31 to 0.10
Broadening	Ratio	1.0	—	—	—
	Tolerance	± 1.0	—	—	—

* Wider tolerance used for this proton is not a result of inaccuracy of NMR data but rather to provide for the fact that the methyl group is not a point proton. The wider tolerance effectively allows for an average over the distance-angle factors for the rapidly rotating methyl group protons.

Table 4 Calculated Average Conformational Angles, Distances and Shift Ratios for AMP and TMP

		AMP					
		H ₈	H ₂	H _{5'}	H _{5'}	H ₁	
<i>r_i</i> (Å)		5.9	11.6	5.6	5.6	8.0	
<i>χ_i</i>		43°	52°	10°	11°	46°	
<i>R</i> _{calc}		0.28	0.00	1.00	1.00	0.08	
		TMP					
		H ₆	H ₅	H _{5'}	H _{5'}	H _{2'}	H _{1'}
<i>r_i</i> (Å)		6.4	7.9	5.4	5.6	8.8	7.5
<i>χ_i</i>		34°	45°	18°	8°	38°	49°
<i>R</i> _{calc}		0.38	0.10	1.00	1.00	0.12	0.07

Distances are measured from the centre of the metal ion which lies 2.9 $\text{\AA}$ from the phosphorus atom. Angles are measured from the symmetry axis of the metal ion which is directed along an axis bisecting the $\text{O}_1\text{-P-O}_3$ angle and lying in that plane.

Table 5a Mean Atomic Coordinates in 0.01 $\text{\AA}$

Atom	AMP			Solution		
	Crystal ¹⁹			0		
Metal				0	0	0
P	-290	0	0	-290	0	0
O_5'	-376	2	136	-376	1	135
C_5'	-296	-20	260	-524	-3	115
C_4'	-390	-8	378	-589	-18	251
C_3'	-530	-70	367	-718	59	280
C_2'	-602	8	481	-717	56	436
C_1'	-551	149	456	-571	87	464
O	-419	135	400	-496	35	353
N_9	-633	225	358	-543	233	471
C_4	-754	284	388	-592	316	570
N_3	-808	298	511	-657	278	682
C_2	-926	358	505	-692	383	755
N_1	-990	397	390	-672	514	721
C_6	-931	393	268	-597	552	614
C_5	-802	330	265	-554	445	530
N_7	-719	295	162	-490	443	408
C_8	-615	235	221	-481	313	377
Torsion angles						
$\text{P-O}_5'-\phi_1$	0°			$-168^\circ \pm 8^\circ$		
$\text{O}_5'-\text{C}_5'-\phi_2$	0°			$-4^\circ \pm 3^\circ$		
$\text{C}_5'-\text{C}_4'-\phi_3$	0°			$100^\circ \pm 5^\circ$		
$\text{C}_1'-\text{N}_9-\phi_4$	0°			$-12^\circ \pm 12^\circ$		

Table 5b Mean Atomic Coordinates in 0.01 $\text{\AA}$

Atom	TMP			Solution		
	Crystal ²⁰			0		
Metal				0	0	0
P	-290	0	0	-290	0	0
O_5'	-371	5	134	-371	4	133
C_5'	-446	-112	179	-503	-58	142
C_4'	-559	-67	272	-542	-74	289
C_3'	-657	28	217	-686	-70	321
C_2'	-710	96	347	-683	-25	470
C_1'	-589	91	441	-557	63	478
N_1	-516	216	454	-581	206	477
C_2	-534	289	568	-620	265	594
N_3	-460	404	579	-637	401	591
C_4	-371	455	482	-623	484	477
C_5	-361	370	365	-584	410	359
C_6	-433	260	356	-568	279	364
Torsion angles						
$\text{P-O}_5'-\phi_1$	0°			$-40^\circ \pm 12^\circ$		
$\text{O}_5'-\text{C}_5'-\phi_2$	0°			$-40^\circ \pm 3^\circ$		
$\text{C}_5'-\text{C}_4'-\phi_3$	0°			$92^\circ \pm 3^\circ$		
$\text{C}_1'-\text{N}_1-\phi_4$	0°			$-24^\circ \pm 5^\circ$		

In the absence of a chelating metal, the phosphate group has three-fold symmetry about the ester bond so that, in the crystal, ϕ_1 torsion angles 120° apart are equivalent. The values quoted above arose because we chose arbitrarily to chelate the metal atom to oxygens O_1 and O_3 (Fig. 4b).

will be useful provided certain conditions are met. It is necessary to have a group on the molecule which will interact with a lanthanide ion. The maximum distance of various protons on the molecule from the complexed ion can be up to 20 $\text{\AA}$ if accurate chemical shifts are measured and high NMR instrumental frequencies used. Several nuclei on the molecule must be affected for an accurate structure to be determined. Finally, computer data analysis is indispensable unless the possible conformations form a trivial set. We believe that this method will be capable of determining the structural properties in solution of a large number of biophysically interesting molecules.

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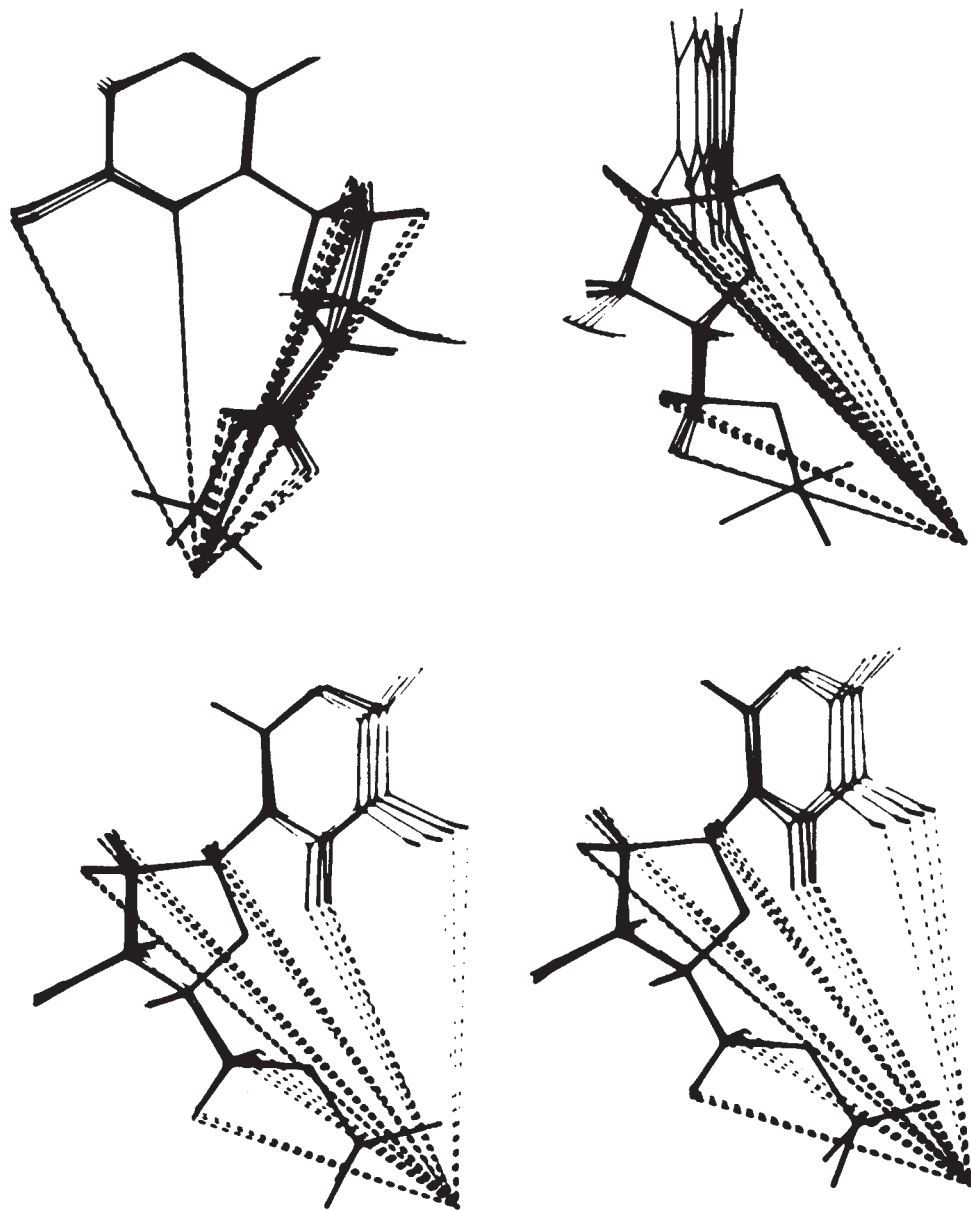


Fig. 8 Family of solution conformations of TMP, orthogonal (top) and stereo views.

Council. We also thank the Medical Research Council for financial assistance.

Note added in proof. Using the above method, the following structure studies have been made: (1) the conformation of several dinucleoside phosphates in water, (2) the conformation of AMP in dimethylsulphoxide, (3) the changes in structure of vitamin B₁₂ derivatives with change of metal ligands, (4) the conformation of ribose-5'-phosphate. In collaboration with Dr J. Feeney, ¹³C shifts and previously unassigned sugar proton data have been used in a more detailed study of the conformation of AMP. The use of the lanthanide probes at high pH has been achieved by complexing with diglycollate. NMR probes for cationic molecules such as acetylcholine are available: [Fe(CN)₆]³⁻, shift, and [Cr(CN)₆]³⁻, broadening. The structures of three intermolecular complexes of metal porphyrins with caffeine, a sterol, and fluorenone have been obtained using transition metal probes. The method can be applied in organic solvents using the europium and gadolinium complexes of acetylacetonate derivatives.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Detection of Radio Emission from Cygnus X-1

THE X-ray source Cyg X-1 is known to be highly variable and recently has been found to pulsate with a period of 73 ms (ref. 1). Here we report the detection of its radio counterpart at a frequency of 1,415 MHz.

Two sets of observations at 1,415 MHz were made with the Westerbork synthesis radio telescope of a $1^\circ \times 1^\circ$ field centred near the position of Cyg X-1. The bandwidth was 4 MHz. Baselines ranging in length from 36 to 1,471 m were used, resulting in a synthesized beam of half-power diameter $23''$ in right ascension and $40''$ in declination. The first observations on February 28, 1971, from 10 h 29 min to 15 h 02 min UT showed no source stronger than 0.005 flux units near the X-ray position. The field was observed again during April 28–29 from 23 h 04 min to 11 h 06 min UT, and in the map resulting from these observations there is an unresolved source within the X-ray error box.

The flux density of the radio source is 0.021 ± 0.004 f.u. and its position, determined by a least-squares fit to the antenna pattern, is in 1950 coordinates: $\alpha = 19$ h 56 min 28.9 s $\pm$ 0.2 s, $\delta = 35^\circ 03' 56'' \pm 3''$. This can be compared with the X-ray position determined by the Uhuru satellite which is $\alpha = 19$ h 56 min 25 s $\pm$ 15 s, $\delta = 35^\circ 03' 25'' \pm 1'$ (unpublished work of Tananbaum *et al.*). Because of the close agreement in position and because this radio source is so strongly variable, it is almost certainly associated with Cyg X-1. A search at the radio position may therefore enable the X-ray source to be identified optically. A likely candidate is the ninth magnitude star AGK2 +35° 1,910 only $1''$ from our position, but photometric and spectroscopic observations are needed to confirm the identification. Further study of the radio emission is also of great importance. Simultaneous measurements over a range of frequencies should reveal whether its radio spectrum is non-thermal as in the case of Sco X-1 (refs. 2 and 3), and observations with high time resolution are essential to determine whether the radio source is a pulsar.

During the preparation of this article we learned from Drs R. M. Hjellming and C. M. Wade that the Cyg X-1 radio source has been detected independently at a frequency of 2,695 MHz with the NRAO interferometer at Green Bank. Their position is in good agreement with that given here.

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Variation of the Strong and Electromagnetic Coupling Constants over Cosmological Times

THERE has been a long history of speculation on the possibility that the fundamental constants of physics may vary slowly over long periods of time^{1,2}. This speculation was most recently brought forward as a possible explanation of the cosmological redshift². Numerous authors pointed out that there are many reasons for ruling out any large scale variations of the fine structure constant (electrical charge) with time³. In this article, we show that on the basis of the liquid drop model of the nucleus and the observed half lives and abundances of the transuranium elements, approximate limits can be put on the rate of change of the ratio g^2/e^2 , where e is the electric charge of the electron and g is the strong coupling constant. If we assume that the strong interactions do not vary as a function of time, then we find limits of the time rate change of e of about the same stringency as those in refs. 3, 4 and 5. If, however, we assume the conclusions^{3–5} that e does not change as a function of time, then we can show that the quantity

$$\frac{1}{g^2} \frac{dg^2}{dt}$$

must be less than $\sim 2 \times 10^{-11} \text{ yr}^{-1}$.

The liquid drop model of the nucleus⁶ treats the stability of the nucleus as the result of the competition between two forces—the Coulomb repulsion between the protons, which tends to expand the nucleus, and the strong interactions, which tend to hold it together. The effect of the strong interactions is introduced through a surface tension T , which we will take to be proportional to g^2 , the strong interaction coupling constant. The criterion that a nucleus of atomic number A and charge Ze be stable against spontaneous fission is

$$Z^2/A < \frac{40 \pi r_0^3}{3 e^2} T \quad (1)$$

where r_0 is the nuclear radius parameter given by $r_0 \sim 1.2 \times 10^{-13} \text{ cm}$.

From this criterion, we see that changes in the ratio T/e^2 could result in a nucleus which was stable becoming unstable and vice versa. For the sake of argument, let us assume that the strong interactions do not vary as a function of time. Then if the redshift is to be explained as an effect of the change of e , e will have been smaller in the past than it is at present. This means that some nuclei which are now unstable would have been stable in the past.

Suppose that we find nuclei which (1) are now unstable, (2) have a long half life, and (3) do not occur naturally. If the electric charge were changing over long time scales, there will be some time in the past, call it t_p , when the electrical charge was such that the nucleus was stable. This time must have been appreciably longer than the half life of the nucleus to allow all the stable nuclei which are present at t_p to have decayed by the present time, and thus meet requirement (3).

We denote by e_p the value of the electric charge at time t_p (the time at which the nucleus was just stable), and e_0 the value of the charge at the present time (t_0). Then

$$\frac{1}{e} \frac{de}{dt} \approx \frac{e_0^2 - e_p^2}{e_0^2 t_p} \quad (2)$$

and the only problem remaining is to assign some value to t_p . From requirement (3) above, we know that $t_p \gg \tau$, the half life of the nucleus. We choose, somewhat arbitrarily, the value

$t_p = 10\tau$, which corresponds to saying that $\sim 0.1\%$ of the nuclei present at t_p will not have decayed by the present time. Given the sensitivity of modern spectroscopic techniques, this is a conservative assignment, and to the level of accuracy of this calculation it is sufficient.

We could determine e_p from the condition

$$Z^2/A = \frac{40 \pi r_0^3 T}{3 e_p^2} \quad (3)$$

if we had a value for T , the surface tension. The standard way of determining T is to look at the semi-empirical mass formula⁶. If we do so, however, we would find that the liquid drop model would predict that many stable nuclei above uranium should exist. In fact, no stable nuclei in that range have yet been discovered. This is because the stability of heavy nuclei depends not only on the relative strengths of the Coulomb forces and surface tension, but on the width of the potential barriers and many other complicated properties of the shell structure of the nuclei. If we say that we can take such effects into account in an approximate way by replacing T in equation (3) by an effective surface tension T_{eff} , then we can determine T_{eff} for nuclei near uranium by noting that ^{238}U is the heaviest stable nucleus. If we assume that ^{238}U is just stable (or just unstable—its half life is about the age of the universe) we find

$$\left. \frac{Z^2}{A} \right|_{\text{U}} = \frac{40 \pi r_0^3}{3 e_0^2} T_{\text{eff}}$$

Substituting this into equation (3), we find

$$\frac{Z^2/A}{(Z^2/A)_{\text{U}}} = e_0^2/e_p^2$$

In Table 1, we give a list of some of the upper bounds on

$$\frac{1}{a} \frac{da}{dt}$$

which we found using several different transuranium elements⁷. The smallest upper bound comes from considering $^{244}\text{Pu}_{94}$, which gives

$$\frac{1}{a} \frac{da}{dt} < 2.3 \times 10^{-11} \text{ yr}^{-1}$$

As the lifetime of the universe is estimated to be 10^{10} yr, this indicates that most of the observed cosmological redshift must come from some source other than changes in the value of e .

A more interesting application of this method comes from observing that bounds on $\frac{1}{e^2} \frac{de^2}{dt}$ of about 10^{-13} yr^{-1} have been set in refs. 3, 4 and 5. We therefore assume that it is the electrical charge which is fixed, and ask what variations in T_{eff} are allowed. Clearly, the values of $\frac{1}{T} \frac{dT}{dt}$ which would result from such a calculation would simply be the negative of the values of $\frac{1}{e^2} \frac{de^2}{dt}$ in Table 1, which were derived by assuming T_{eff} to be a constant.

Although T_{eff} will, in general, have a very complicated dependence on the strong interaction coupling constant g^2 , a reasonable first approximation is to assume that $T \propto g^2$, so that

$\frac{1}{T} \frac{dT}{dt} = \frac{1}{g^2} \frac{dg^2}{dt}$. In this case, the smallest upper bound on the time variation of the strong interaction strength is

$$\left| \frac{1}{g^2} \frac{dg^2}{dt} \right| < 2.3 \times 10^{-11} \text{ yr}^{-1}$$

The argument based on the stability of the heavy elements shows that if g is constant, e cannot have been much less in the past than it is now, and if e is constant, then g cannot have been stronger in the past than it is now. This latter result has not yet, to our knowledge, been pointed out.

Table 1 Upper Bounds on $\frac{1}{a} \frac{da}{dt}$

Element	Half life (yr)	$\frac{de^2}{e^2 dt} = -\frac{dg^2}{g^2 dt} (\text{yr}^{-1})$
$^{247}\text{Bk}_{97}$	1.3×10^3	5.2×10^{-6}
$^{244}\text{Pu}_{94}$	7.6×10^7	2.3×10^{-11}
$^{237}\text{N}_{93}$	2.14×10^6	1.2×10^{-9}
$^{247}\text{Cm}_{96}$	1.6×10^7	3.0×10^{-10}
$^{250}\text{Cm}_{96}$	1.7×10^4	2.1×10^{-7}

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Production of Proton-rich Nuclei

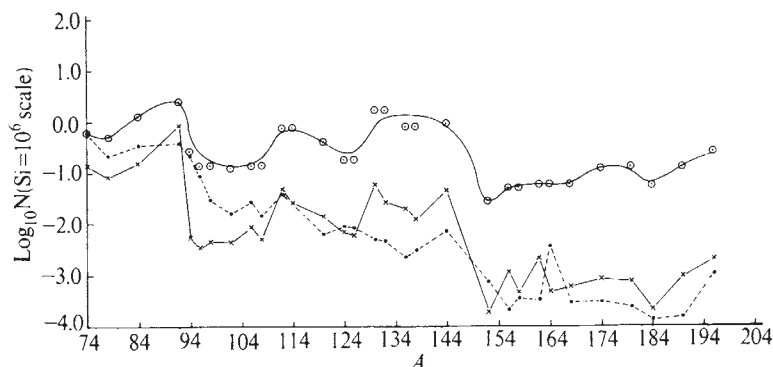
THE p-elements, those rich in protons, are less abundant than the s or r-elements by factors between 10^{-3} and 10^{-4} , and are usually bypassed in a slow or fast neutron capturing chain. It has been suggested that they are produced either by (p, γ) or (γ , n) reactions¹. Formation by (p, γ) reactions in the envelopes of supernovae, however, would be limited by the short duration of the phenomena². Other suggestions by Ito³ and Malkiel⁴ are also inadequate. The smoothness of the abundance distribution of p-elements^{5,6}, however, suggests that they might have been synthesized in one physical environment, and recently there have been attempts to explain the abundances by positron capture in s-elements⁷ or by spallation processes⁸. This article is a preliminary report of an investigation of the abundance distribution of p-elements formed in a single event—the fission of highly excited nuclei in supernovae.

I assume that highly excited compound nuclei such as ^{254}Cf , ^{254}Fm are produced by the bombardment of Th, U (possibly already produced in a rapid process) by ^{12}C , ^{16}O ions. Recent experiments⁹ indicate that the de-excitation of the compound nuclei occurs almost exclusively by neutron evaporation before fragmentation of the fissile nuclei. Because the kinetic energy of the fragments is due only to Coulomb repulsion, the high excitation energy given to the target nucleus by the incident ion, accelerated at some stage in the supernova conditions, can be used in processes other than projection of the fission fragments. It is most likely that the dissipation of this energy is by the evaporation of large numbers of neutrons. But to limit the increase of Z^2/A , one or two protons or one alpha particle often seem to be evaporated per ten neutrons⁹. Assuming that the nuclei are excited to energies > 100 MeV such that about twenty neutrons are evaporated before fission, the fission fragments will naturally be mostly proton-rich.

The independent fission yield $P(A, Z)$ of a particular p-element is then obtained from¹⁰

$$P(A, Z) = (\pi C)^{-1/2} \exp \left(-\frac{(z - z_p)^2}{c} \right) \quad (1)$$

Fig. 1 The relative fission yields of p-elements after neutron capture in supernova (upper curve). The solid line connecting the crosses shows the p-element abundances adjusted to ^{114}Sn observed abundances after photodisintegration. The observed abundances are shown by the dotted curve.



where $C=0.9$ and Z_p is the charge number which is formed with the highest yield for a given mass A . The value of Z_p is obtained from

$$Z_p = A/(1.98067 + 0.01496 A^{2/3}) \quad (2)$$

where A is the mass number under consideration. I have considered seven fissile nuclei: ^{254}Cf , ^{256}Cf , ^{254}Fm , ^{256}Fm , ^{258}Fm , ^{260}No , and ^{264}No , chosen because their lifetimes against spontaneous fission range from a few hours to about 100 days, like the half period of a complicated supernova light curve.

As the p-elements are produced, they are exposed for a short time to a large flux of neutrons in a rapidly expanding situation, so they are slightly modified by neutron absorption processes. The neutron-rich elements are not produced to any significant extent, so neutron absorption in those nuclei need not be considered. The probability of neutron absorption is given by $(P_A \sigma_A / \sum P_A \sigma_A)$ and the fraction of p-elements left behind is given by

$$P_A / (P_A \sigma_A / \sum P_A \sigma_A) \quad (3)$$

where σ_A is the neutron absorption cross-section in A . The summation is taken over the whole range of p-elements, and I have taken the cross-section data from Burbidge *et al.*¹ for these nuclei. The yields are then normalized to the $Z=34$, $A=74$ abundance from Cameron⁶. This is shown by the upper smooth curve in Fig. 1.

The p-elements left behind are then substantially modified by the radiation at very high temperatures. Recently Macklin suggested¹¹ that the p-elements might be produced by the (γ, n) reaction because there seemed to be a strong correlation between p-process abundances and neutron binding energy. The probability of photodisintegration is dominated by the term $\exp(-B_n/kT)$, where B_n is the neutron binding energy. The p-element yields are modified according to the value of the neutron binding energies in these nuclei. In the present case, the first step is to bring the final yield of ^{114}Sn to the observed value and then calculate the relative final yields of all other p-elements from $A=74$ to $A=196$. These are shown by the solid lines joining the crosses in Fig. 1. The dotted curve is a plot of the observed abundances due to Cameron⁶ for comparison. Data on the binding energy of the last neutron come from Mattauch *et al.*¹². In a few cases where data were not available I took values from Macklin's work¹¹.

Except for a few cases, the calculated and observed values are in very satisfactory agreement. Thus it may be surmised that several complicated processes may be at work in supernovae to produce all the p-elements in one physical environment.

The nuclei undergoing fission and giving rise to the abundance distribution of p-elements must be highly excited. This is possible only when they are produced by accelerated ions. The main contributor to the fission yield is found to be ^{254}Fm , and this is not a product of a rapid neutron capture chain. Thus there is no conflict with the idea that ^{254}Cf is produced in type I supernovae through rapid neutron capture¹³. But ^{254}Fm and

other such nuclei which will be produced in conditions of high excitation may also contribute to the energy output of the complicated supernova light curve. It may be necessary to examine the whole problem of the synthesis of heavy elements in the transuranic region through heavy-ion reactions. But I should add that the phenomenon of high energy fission also requires detailed investigations, both theoretically and experimentally, before any definitive conclusion can be reached.

My use of the data on thermal neutron absorption cross-sections may not be justified because the fission neutrons are emitted with much more higher energies. But, except for resonances, there may not be large relative variations in the cross-sections even at higher energies.

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Magnetospheric Electric Fields and the Super-rotation of the Earth's Upper Atmosphere

SEVERAL mechanisms have been proposed to explain the super-rotation of the Earth's upper atmosphere discovered by King-Hele¹. These mechanisms can be divided into two general groups. The first considers the absorption of solar radiation within the upper atmosphere as the original driving force for the super-rotation, whereas the second assumes the primary source to lie outside the upper atmosphere. The first group can be subdivided into three parts which give differing hypo-

theses to explain the transformation of solar radiation energy into kinetic energy of the super-rotation.

First, King-Hele¹ considered the geostrophic component of the zonally averaged longitudinal wind as the prevailing wind which he observed. Such wind can be set up by pressure gradients directed towards the poles as the result of the net surplus of solar heat input into the lower latitudes as compared with the heat input into the polar regions. Hines² has pointed out, however, that this wind component must be rather small due to the large ageostrophic wind component which results from ion-neutral collisions. Quantitatively³, confirmation of this results in a prevailing west wind of the order of 5 m s^{-1} at F2 layer heights which is at least one order of magnitude smaller than the winds suggested by King-Hele derived from satellite drag data⁴. Moreover, that wind component is zero at the equator which is inconsistent with the findings of King-Hele.

Second, super-rotation (or prevailing west winds) may be set up by non-linear coupling between the diurnally varying pressure and density fields originating from the solar heat input⁵⁻⁷. Here, prevailing west winds blow only within the lower latitudes ($|\phi| < 30^\circ$) while at middle and high latitudes the prevailing wind component changes into an east wind.

Third, nonlinear coupling may occur due to a diurnally varying impulse exchange between neutrals and ions where the electric polarization field of the Sq region acts as a linking mechanism⁵. This effect, however, seems to be insignificant for the generation of a prevailing wind component⁷. Rishbeth⁸ proposed a new coupling mechanism between neutrals and ions at F layer heights which should result in a prevailing west wind. Here, the linking chain is an electric polarization field of (what he calls) the F layer dynamo. This mechanism seems to work only if the dynamo region of the E layer is dynamically decoupled from the F layer. It can be shown, however, that the predominant wind system at E layer heights which drives the Sq current belongs to the same tidal mode as the wind of the F layer derived by Kohl and King⁹ and by Geisler¹⁰. That mode propagates through the E and the F regions continuously increasing in magnitude and only slightly changing its phase and latitudinal structure³.

Following an idea of Cole¹¹, we want to discuss a hypothesis which belongs to the second class of driving mechanisms. It is a zonally averaged electric field of magnetospheric origin observed by Mozer and Manka¹² at balloon altitudes. This electrostatic field can be derived from a potential (my unpublished results)

$$\Phi = \frac{r_0 A \sqrt{\rho}}{\sin \theta} \left(\rho \geq 1; \theta \neq \begin{cases} 0 \\ \pi \end{cases} \right) \quad (1)$$

with $\rho = r/r_0$, r = distance from the Earth's centre, $r_0 \sim 6,570 \text{ km}$ (upper boundary of the dynamo region), θ = polar distance and $A = 3.5 \text{ mV m}^{-1}$.

The electric field strength components at F layer heights and above and outside the polar regions are

$$\begin{aligned} E_\theta &= \frac{A \cos \theta}{\sqrt{\rho} \sin^2 \theta} \\ E_r &= -\frac{A}{2 \sqrt{\rho} \sin \theta} \end{aligned} \quad (2)$$

The plasma of the F layer drifts within the combined electric and geomagnetic fields like

$$\mathbf{v}_i = \frac{\mathbf{E} \times \mathbf{B}}{B^2} \quad (3)$$

where B is the geomagnetic induction. In the case of an ideal co-axial geomagnetic dipole field, equations (2) and (3) result in a prevailing eastward drift of the ions of

$$(v_i)_\lambda = \frac{A}{2 B_0 \sqrt{\rho} \sin^2 \theta} \left(\rho \geq 1; \theta \neq \begin{cases} 0 \\ \pi \end{cases} \right) \quad (4)$$

where B_0 is the geomagnetic induction at the equator. At F2 layer heights ($\rho \sim 1$) and within the equatorial plane ($\theta = \pi/2$) this velocity is $(v_i)_\lambda \sim 60 \text{ m s}^{-1}$ increasing with latitude.

The neutral air at F layer heights in the absence of external forces is dragged by the ionospheric plasma according to

$$v_\lambda \sim \frac{v_c}{v_\nu + v_c} (v_i)_\lambda \quad (5)$$

where v_c is the non-neutral collision number and v_ν is a measure for the molecular viscosity force acting on the neutral gas³. Since v_c decreases like the electron density with altitude above the maximum of the F2 layer, and since $v_\nu < v_c$ at F layer heights, we expect from equations (4) and (5) a prevailing west wind of the neutral component of the air which has its maximum at F layer heights. It has the right direction and the right order of magnitude to be consistent with the observations of King-Hele.

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Lunar Electrical Conductivity Profile

RECENT measurements and analysis of magnetic field fluctuations on the surface of the Moon have led Sonett *et al.*¹ to conclude that the Moon is stratified into an upper mantle, a lower mantle and a core. Using an eight-layer model, their computer analysis of magnetic field fluctuation data led to the conclusion that there is a highly conducting shell about 100 km thick, located about 220 km below the lunar surface. In this article their experimental data are reanalysed in terms of a simple two layer model. I conclude that the field fluctuation spectrum observed accurately follows from a Moon with an inner core which has a uniform conductivity of $6 \times 10^{-4} \text{ mho m}^{-1}$ and an insulating mantle about 160 km thick. These results coincide more closely with the preliminary conclusions of Sonett *et al.*² than their more refined calculations which indicate a very non-uniform and non-monotonic conductivity profile.

My analysis follows all the basic assumptions of Sonett *et al.*¹ including the neglect of toroidal fields generated by the Moon as a result of induced currents passing through the lunar crust. We assume a spatially uniform exciting field, and so will restrict our attention to magnetic fields with dipole symmetry in angle.

For the purpose of orientation it is instructive to consider first a very simple model—that of a Moon with a perfectly conducting core of radius r_0 , and an insulating mantle out to its surface at r_1 . Outside the Moon there is a perfectly conducting plasma. A uniform magnetic exciting field at infinity $B = B_0 \hat{z} e^{i\omega t}$ leads to the boundary condition at r_1 that $B_r = B_0 \cos \theta e^{i\omega t}$. I will use the usual spherical coordinates r, θ, ϕ , MKS units with a Coulomb gauge vector potential $A(B = \nabla \times A, \nabla \cdot A = 0$ and $E = -\partial A / \partial t - \nabla \Phi)$.

To treat the problem we need to introduce a vector potential with only one component, A_ϕ , and no electrostatic potential Φ . In the insulating mantle A_ϕ is of the form

$$A_\phi = A_1 \sin \theta \left(r - \frac{A_2}{r^2} \right) e^{i\omega t} \quad (1)$$

with A_1 and A_2 being arbitrary constants. Noting that $A=0$ at r_0 , we obtain for the amplification factor A_0 , that is, the horizontal field B_θ at $\theta=\pi/2$ divided by the exciting field B_0

$$A_0 = \frac{1 + \frac{1}{2} \left(\frac{r_0}{r_1} \right)^3}{1 - \left(\frac{r_0}{r_1} \right)^3} \quad (2)$$

The next level of sophistication which I shall consider is that of a Moon with an insulating mantle and a core of uniform conductivity σ out to a radius r_0 . In the mantle region between the core and the lunar surface the vector potential is given by equation (1). We must join this vacuum solution on to the solution A_ϕ for a core of finite conductivity, at $r=r_0$. Reducing the differential equation for A , that is $\nabla \times \nabla \times A = -\mu_0 \sigma (\partial A / \partial t)$ to spherical coordinates with A_ϕ of the form $A_\phi = A'(r) \sin \theta e^{i\omega t}$ we obtain

$$\frac{1}{r} \frac{\partial^2}{\partial r^2} (rA') - \frac{2A'}{r^2} - i\omega\mu_0 \sigma A' = 0 \quad (3)$$

It is most convenient to normalize r to the skin depth in solving this equation, that is, $y=r/\delta$ ($\delta \equiv \sqrt{2/(\omega\mu_0\sigma)}$). The solution to (3) which remains finite as $y \rightarrow 0$ can be written ($\sqrt{2i} \equiv 1+i$)

$$A'(y) = \frac{e^{\sqrt{2i}y}}{y} \left(1 - \frac{1}{\sqrt{2i}y} \right) + \frac{e^{-\sqrt{2i}y}}{y} \left(1 + \frac{1}{\sqrt{2i}y} \right) \quad (4)$$

To join the solution of A in the core to that in the mantle we require both B_r and B_θ to be continuous at $r=r_0$, that is at $y_0 \equiv r_0/\delta$. Introducing the function

$$\alpha(y) = \frac{1}{A} \cdot \frac{\partial}{\partial y} (yA') \quad (5)$$

and $\alpha_0 \equiv \alpha(y_0)$ we obtain for the horizontal field amplification

$$A = \frac{1 + \frac{1}{2} \left(\frac{r_0}{r_1} \right)^3 \left(\frac{\alpha_0 - 2}{\alpha_0 + 1} \right)}{1 - \left(\frac{r_0}{r_1} \right)^3 \left(\frac{\alpha_0 - 2}{\alpha_0 + 1} \right)} \quad (6)$$

This quantity is complex; its magnitude is plotted in Fig. 1. The magnitude of A described by (6) is quite accurately obtained from the perfectly conducting model by replacing r_0 in (2) by $r_0 - \delta/2$ provided that y_0 is greater than about 3.

The experimental data of Sonett *et al.* have been plotted in Fig. 2 together with the best fit curve derived from this two layer, finite conductivity theory with the outer 160 km of the Moon insulating ($(r_0/r_1)^3 = 0.75$) and the inner core with a conductivity of 6×10^{-4} mho m^{-1} . We note that the fit obtained is much better than we should expect from such a simple model.

In fitting this two layer model to the data, several trends are evident. The fact that the amplification is fairly independent of frequency between 0.01 and 0.04 Hz leads to the conclusion that there is an insulating crust on the outside of the Moon, that is the electrical skin depth of the outer crust at, say, 0.025 Hz must be large compared with the crust thickness. According to equation (2) an amplification factor of 4 is obtained from a perfectly conducting lunar core the outer surface of which is 220 km below the lunar surface, or from a field penetration of 220 km into the Moon. In this range of amplification the field penetration and amplification are inversely related to each other.

The knee in the amplification against frequency curve occurs when the variation in the depth of field penetration into the core becomes the dominant factor in determining the amplifica-

tion. We have already noted that the total effective field penetration depth of a finite conductivity core theory is the thickness of the insulating crust plus one half the core's electrical skin depth. At 0.01 Hz and 6×10^{-4} mho m^{-1} , one half the skin depth is 100 km—thus the total field penetration depth is, including the 160 km crust, 260 km. The amplification predicted below 0.01 Hz falls off because, at lower frequencies, the effective penetration depth is determined by variation in field penetration into the core; at higher frequencies, the amplification is relatively constant because the effective penetration depth is determined mostly by the thickness of the insulating crust.

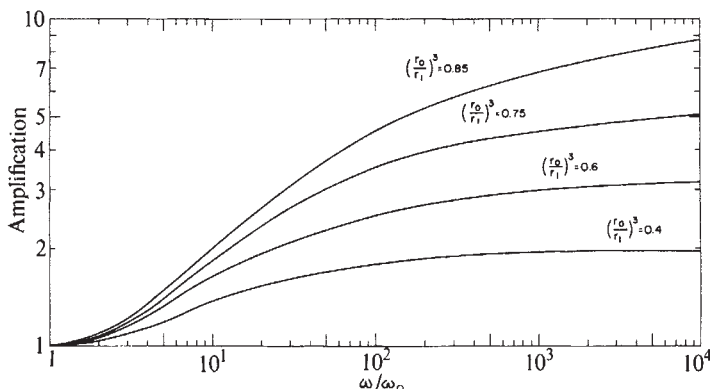


Fig. 1 Horizontal field amplification against normalized frequency ω/ω_0 ($\omega_0 \equiv 2/(\mu_0 r_0 \sigma)$) for the two layer model considered.

The fact that the observed amplification falls off with frequency so closely to that predicted by this simple two layer model is an indication that the conductivity of the core remains fairly constant below the transition depth of 160 km. To estimate the effect of a further rise in conductivity with depth we have considered the response of a three layer Moon by juxtaposing two of the response curves shown in Fig. 1. The frequency response of such a three layer Moon, with high conductivity in the inner core, can be estimated by noting that, at low frequencies, the important currents flow in the inner core and, at higher frequencies, in the outer one. A Moon with an inner core with conductivity of 2×10^{-3} mho m^{-1} to 1,360 km radius covered by a layer with a conductivity of 6×10^{-4} mho m^{-1} to 1,580 km radius produces a response curve which distinctly falls off more slowly with frequency than is observed. The observation that an amplification of 1.2 is maintained for 15 min (2×10^{-4} Hz) on the rear side of the Moon after a magnetic "step" excitation³ indicates that there is some rise in conductivity with depth.

The fact that the knee in the experimental frequency amplification characteristic of the Moon is so distinct indicates that the insulating mantle-conducting core transition is quite sharp. Using similar reasoning as above we conclude that the conductivity at the outer edge of the conducting core must fall off by at least an order of magnitude in less than 50 km. The greatest uncertainty in the theory results from the fact that experimental data³ indicate that the rear side of the Moon is not enveloped by a conducting plasma as is the front. This has the effect of reducing the field amplification, that is, this theory will overestimate the thickness of the outer crust. Also this model will slightly overestimate the conductivity of the core.

The fit of the two layer model is not unique and this is evident by the close coincidence of the predicted spectrum when compared with that resulting from the very different conductivity profile which Sonett *et al.* propose. They have also noted that their computer program always generates a peaked profile to fit the data. They never find anything resembling a simple two layer model as I propose. Their computer program apparently selects an optimum conductivity at $r=800, 1,200, 1,400, 1,450, 1,490, 1,510, 1,550$ and $1,740$ km and linearly interpolates $\ln \sigma$ in between. Such a routine is basically unable

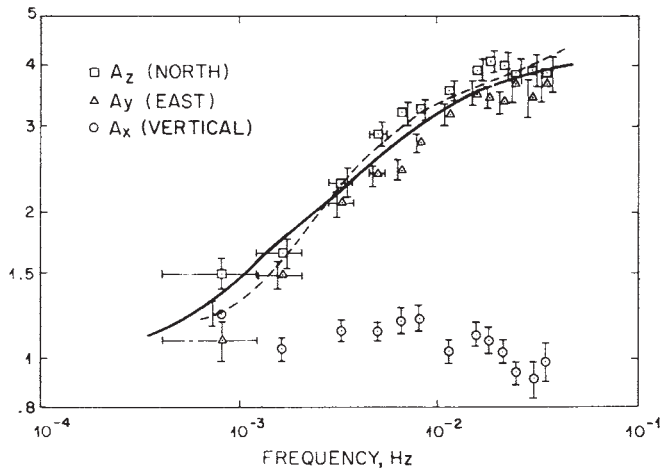


Fig. 2 Experimental field amplification data and theoretical fits. Dashed line represents the eight layer theory of Sonett *et al.*¹. Solid curve represents the best fit to two layer theory, $\sigma = 6 \times 10^{-4}$ mho m^{-1} , $(r_0/r_1)^3 = 0.75$ ($r_0 = 1,580$ km).

to optimize and generate the simple profile I propose. The flatness of the response at high frequencies forces the computer to choose a low conductivity at both 1,550 and 1,740 km. Then to make the amplification factor equal 4 by adjusting the conductivity at 1,510 km requires a very high conductivity value. There is no possibility left of allowing much field penetration into the core because a perfectly conducting core surface at 1,520 km is needed to produce an amplification of 4. Once a very high conductivity value at 1,510 km is chosen the program must "compensate" by hollowing out the conductivity at smaller radii.

Though the profile which has been derived on the basis of a simple two layer Moon is not unique the results suggest several basic conclusions. The outer 160 km of the Moon is highly insulating. The conductivity in the mantle transition layer decreases at least an order of magnitude in 50 km or less from about 6×10^{-4} mho m^{-1} . Finally, the conductivity remains reasonably constant between 160 and 360 km depth. A conductivity rise to more than 2×10^{-3} mho m^{-2} at a depth of 360 km is not compatible with the data when analysed in the context of this model.

The data are well fitted by this simple two layer model, and so the conclusion, based on magnetic fluctuation data, that the Moon has a stratified composition is less justified.

I thank Dr D. L. Turcotte for helpful discussions.

Addendum. Blank and Sill⁴ and Schubert and Schwartz⁵ have also considered the response of a two layer Moon to magnetic fluctuations. Indeed, relation (6) can be derived from the work of Schubert and Schwartz⁵. Sonett *et al.*⁶ considered and rejected a two layer Moon with almost identical parameters as proposed here because it was concluded that such a model does not properly fit the low frequency observations (10^{-3} Hz) and does not change curvature at high frequencies (2×10^{-2} Hz) as the observations indicate. The lack of fit at high frequencies occurred because of the low frequency approximation used in their calculations. Unfortunately, the experimental data at low frequencies are not adequate to discern between the eight layer model and the two. At 8×10^{-4} Hz particularly, the difference between the north-south and east-west observations and the deviation of the vertical amplification from unity are very important considerations. Though the eight radii model quoted¹ cannot optimize to the two layer model above, Sonett *et al.* also studied other combinations of radii and conductivity with their computer routine; they, however, always find a peaked conductivity profile as giving the best fit as stated in their original article.

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Convection and the Constant Q-Mechanism

It has been argued¹ that the data on the Q value of the Earth's mantle do not militate against convection currents because the range of frequency over which Q has been proved constant in the laboratory ($10^{-4} < f < 10^6$ Hz) does not lie entirely within that which can be explained by the Lomnitz law² of creep in rocks ($10^{-8} < f < 1$ Hz). The Lomnitz law is characterized by a decreasing rate of creep that forbids convection and McCann rightly points out that according to this law the mechanism which accounts for the constant Q over the laboratory proven range is inexplicable and so convection is, in fact, allowed as the law is not representative of rocks thought to make up the Earth's interior. There are several comments to be made on this reasoning. (i) The processes responsible for damping in the mantle are not the same as those dominant in the laboratory. Experiments made at atmospheric pressure are dominated by microcracks, as noted by Anderson³ and Orowan⁴. These cracks are closed by pressures of only 2 or 3 kbar, corresponding to a depth of approximately 10 km. Thus, laboratory data that show a wide range of frequency-independent Q are not of any use at depth unless special experimental arrangements are made. (ii) Most experiments on damping are inherently unsatisfactory because they are made on the width of the damping peak in amplitude, that is liable to be broadened by numerous effects (ref. 2, page 334). (iii) Lomnitz⁵ in his original work used a torsion method to investigate the anelastic response of rocks to a suddenly applied stress, P , and arrived at the law

$$\epsilon = \frac{P}{\mu} [1 + q \log(1 + at)]$$

where ϵ is the strain at time t , μ is the rigidity, and q , a are constants. It is an empirical creep law, and needs connecting with an explicitly stated damping process before any fundamental connexion between the two can be attempted⁶. Indeed, there may well be no connexion between the creep processes and the damping processes operative in the Earth, as they are rate-controlled in different ways on an atomic scale.

In considering the above points, it must be stated that the Lomnitz law has in its favour the fact that it can explain the range over which Q has been found constant in the Earth (11–25 s periods). Whether it is valid to use it in explaining this constancy is another matter, and one that is open to doubt. The modification of the Lomnitz law (ref. 2, page 12) explains many phenomena dependent on the anelastic response of the Earth's interior, among which are (a) the relaxation times for the Moon's librations, (b) the secular acceleration of the Moon, (c) the rotation of the Moon, (d) the change of the Moon's ellipticities and (e) the compensation of second order and other harmonics in the gravity field. It also helps in explaining the rotation of Mercury and various small bodies of the solar system; and the data on Q must be examined in the light of these successful applications of the law. In contradistinction, the elasticoviscous law leads to some unacceptable conclusions, notably its prediction that mountains and other irregularities on the surface of the Earth should have subsided from sight long ago.

If convection in the upper mantle is valid, a compromise between the Lomnitz or modified Lomnitz law and the desirable

consequences of the elasticoviscous law might be made in the sense of Murrel⁷ and others: a lower mantle obeying the former type of logarithmic creep law could be responsible for the effects (a) to (e) mentioned here, while a convecting upper mantle would allow acceptance of the data from regional uplift following deglaciation as regards the viscosity of the upper 700 km. The characteristic relaxation time from the study of Fennoscandia (10^7 – 10^9 s) is in approximate agreement with that of the Chandler wobble damping (10^8 s); if these rough values could be improved on it might be worthwhile to see if the damping of the Chandler wobble could be accomplished in the upper 700 km layer, the boundary between the upper and lower mantle being taken at this depth on seismic and phase change bases. While the Fennoscandian viscosity of 10^{20} – 10^{23} poise allows continental drift with a convective driving force, there is some case to be made for the impossibility of classical drift⁸ and it may transpire that a two component mantle allows convection in the upper region which is, however, decoupled from the crust or otherwise unable to cause continental drift.

I thank Sir Harold Jeffreys for discussion.

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Occurrence and Geological Significance of Layered Stratiform Intrusion in the Yilgarn Block, Western Australia

I WISH to announce the presence in the Yilgarn Block¹, Western Australia, of a major stratiform basic igneous complex with many features similar to the well documented Bushveldt², Skaergaard³ and Stillwater layered⁴ intrusions. There is a stratiform basic sill 64 km south-east of Mount Magnet within the complexly deformed Archaean greenstones of the Yilgarn Block⁵. The sill, here provisionally designated the Challa Intrusion, crops out over an area in excess of 400 km², with a discontinuous strike length of more than 200 km in a north-westerly direction from Challa through Barrambie to Yarrabubba, 30 km south of Meekatharra (Fig. 1). The separate occurrences of vanadium-rich titaniferous magnetites that outcrop along the whole of the sill from Youanmi in the south through Wyandoo, Windimurra, Challa, Windsor and Barrambie to Yarrabubba in the north, have not been recognized before as part of the one deposit. The sill consists of alternating layers of gabbros, anorthosites, pyroxenites and magnetite layers and lenses; the principal rock type is a medium to coarse grained gabbro with an average grain size of approximately 4 mm.

Because of the absence in most areas of a basal ultrabasic zone and also because of the intrusion of "younger granite"⁶ into the layered basic rocks, the true thickness of the sill is difficult to establish. At Challa, a thickness of 800 m has been measured, and at Barrambie a thickness of 1,700 m (unpublished results of R. J. Korsch). To the east of Challa where the strike of the sill is parallel to that of the adjacent chlorite schists and a banded iron formation, gabbroic and pyroxenitic layers predominate. To the west anorthosite layers become prominent whereas the central portion of the sill is marked by alternating layers of mafic and feldspathic gabbro associated with up to twelve magnetite layers and lenses ranging from a few millimetres to a few metres thick, that are extraordinarily persistent

along the strike. The magnetite layers and lenses consisting of individual magnetite crystals up to 3 cm long have been found to contain fairly high concentrations of vanadium (up to 1.96% V₂O₅) and titanium (up to 21% TiO₂). Gabbros from the central portion of the sill invariably contain some disseminated magnetite, with up to 0.5% V₂O₅.

An unusual feature of some pyroxenitic layers from the lower portion of the sill is the development of pyroxene crystals up to 10 cm long. The rocks from the ultrabasic zone outcropping in the vicinity of Mulyron Hill and Narndee are characterized by the presence of disseminated nickel sulphide.

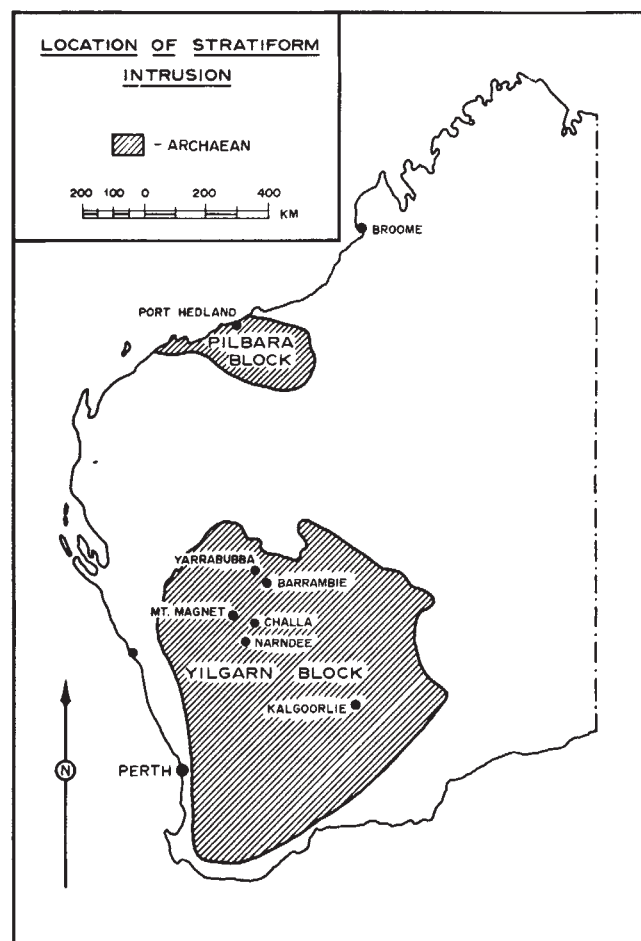


Fig. 1 The location of the Yilgarn Block.

As in many stratiform intrusions⁷ not all the monomineralic layers of the Challa Intrusion show cumulate textures indicating that processes other than gravitative settling may have been operative during crystallization, which occurred in conditions of high oxygen fugacity, indicated by the fact that most of the iron has been precipitated as ferric oxides.

The significance of the discovery of a layered intrusion within the Archaean rocks of Western Australia exhibiting similarities in structure, petrology and mineralogy to that of the Bushveldt Complex of South Africa⁸ is that it may have widespread economic as well as geological implications. Investigations by several exploration companies are in progress to determine the size, grade and economic potential of the nickel and vanadium ore deposits from the lower and central portions of the sill respectively. Within the central portion of the sill the possibility of finding chromite mineralization associated with the stratigraphically lower magnetite layers and lenses as well as platinum-group minerals in the adjacent gabbros should not be discounted. Mineralogical, petrological and petrochemical studies of the Archaean rocks of Western Australia will be

stimulated by the occurrence of a layered stratiform basic intrusive, within the Yilgarn Block.

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Estimation of Nuclear Explosion Energies from Microbarograph Records

FOLLOWING the US and USSR atmospheric test series in 1954–1962, numerous microbarograph records^{1–8} of air waves generated by nuclear bomb tests were published. Previous theoretical interpretations^{7,9} of such waveforms have required some explicit knowledge of the average atmospheric temperature and wind profiles above the path connecting source to microbarograph. Such profiles are never sufficiently well known and vary from point to point, and as seemingly small changes in the profiles cause relatively large changes in the waveforms, it would seem to be difficult to estimate the explosion energy yield to even order of magnitude accuracy from such records. Recently, however, in a further account of this work to be published elsewhere, we have succeeded in deriving an approximate theoretical relationship between certain waveform features and energy yield which is insensitive to changes in atmospheric structure. This relationship is given by

$$E = 13 p_{FPT} [r_e \sin(r/r_e)]^{\frac{1}{2}} H_s (c T_{1,2})^{\frac{3}{2}} \quad (1)$$

where E is energy release, p_{FPT} is the first peak to trough pressure amplitude (see Fig. 1), r_e is radius of the Earth, r is the great circle distance from burst point to observation point, H_s is a lower atmosphere scale height, c is a representative sound speed, and $T_{1,2}$ is the time interval between first and second peaks. The purpose of the present communication is to describe the extent to which the above relation agrees with the existing available data.

The various points shown in Fig. 1 correspond to individual microbarograms recorded at Pasadena, California; Berkeley, California; Terceira, Azores; Fletcher's Ice Island; Whipsnary, New Jersey; Ewa Beach, Hawaii, and Palisades, New York, after the Soviet explosions of (a) September 10 (10 MT), (b) September 11 (9 MT), (c) September 14 (7 MT), (d) October 4 (8 MT), (e) October 6 (11 MT), (f) October 20 (5 MT), (g) October 23 (25 MT), (h) October 30 (58 MT), and (i) October 31, 1961 (8 MT) and the US explosions of (j) May 4 (3 MT), (k) June 10 (9 MT), (l) June 12 (6 MT), (m) June 27 (24 MT), and (n) July 11, 1962 (12 MT). Here the estimate of the yield (in equivalent megatons of TNT where one MT equals 4.2×10^{22} ergs) is taken from Båth¹⁰. All the records used are taken from the articles of Harkrider⁷ and of Donn and Shaw⁸. Pressure amplitudes for Harkrider's records were computed using his microbarograph response data. Pressure amplitudes for the Donn and Shaw records were determined according to the premises (W. Donn, private communication) that (a) all records recorded by Lamont type A microbaro-

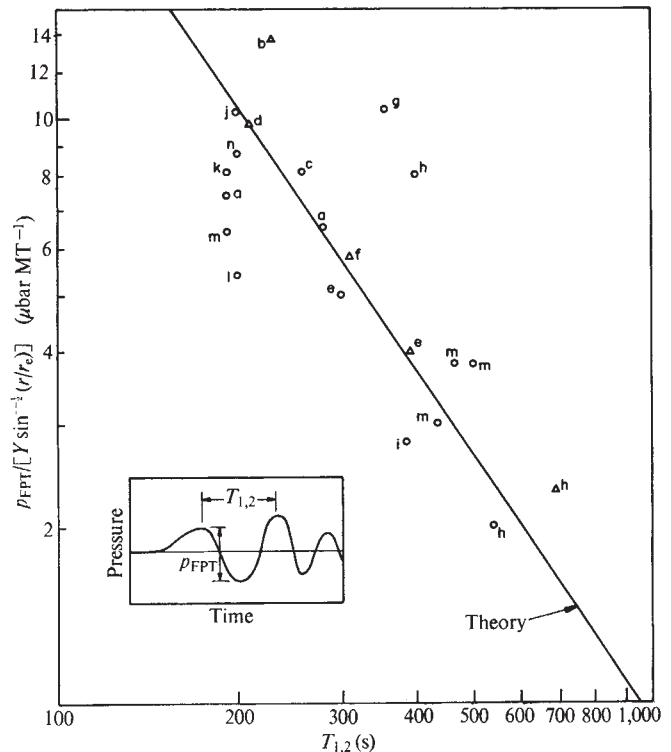


Fig. 1 Comparison of data with the theoretical relationship between amplitude and period of infrasonic waveforms generated by nuclear explosions. The data points are lettered a to n corresponding to particular events defined in the text. $\circ$, Donn and Shaw; Δ , Harkrider.

graphs are to the same scale and (b) the clip to clip amplitude of off scale oscillations was 350 μ bars. The ordinate in Fig. 1 gives $p_{FPT} [Y \sin^{-1/2}(r/r_e)]$ in μ bar MT^{-1} where Y is the explosion yield in MT. The abscissa gives the period $T_{1,2}$ in s. Note that the plot is full logarithmic. The solid line represents the theoretical relation, equation (1) with c and H_s taken as 310 $m s^{-1}$ and 8 km, respectively.

The scatter about the theoretical curve could be due to various causes; one which seems especially likely is the undulation in amplitude due to the horizontal refraction and subsequent focusing or defocusing caused by departures of the atmosphere from perfect stratification. We may note also that much of the scatter would not be present if we had omitted data corresponding to explosions of greater than 11 MT. The general trend of longer period signals being of lower amplitudes than signals recorded elsewhere but which were generated by the same event seems to be amply substantiated by the data.

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BIOLOGICAL SCIENCES

RNA-dependent DNA Polymerase from *E. coli*: an Effect produced by Traces of Added DNA

WITH an enzyme from *Escherichia coli* containing both DNA and RNA dependent DNA polymerase activities, we have found that RNA and DNA together have a synergistic effect on DNA synthesis.

It has been shown^{1,2} that oncogenic RNA viruses contain an enzyme or enzymes capable of synthesizing DNA *in vitro* using the virus RNA as a primer or template. The flow of information from RNA→DNA had also been shown to occur *in vitro* using enzymes derived from *E. coli*³ where poly rA·rU was shown to serve as a template for the synthesis of poly dA·T. Poly C·I was also shown to produce poly dC·rG or poly rC·dG, depending on the substrates used⁴; the hybrid product poly rA·T also served as a primer in the latter system. These synthetic polymers can also be used by the enzymes of the oncogenic RNA viruses⁵. We have shown that natural RNAs, specifically rat liver 5S and ribosomal RNAs, also serve as primers or templates *in vitro* in the *E. coli* DNA polymerase system⁶. Although it is possible that these RNAs are contaminated with DNA in minute amounts, there can be no contamination in the case of the synthetic polynucleotide. It seems probable that both the ribohomopolymers and the natural ribonucleic acids do catalyse a reaction *in vitro*. However, we regard the question of DNA contamination as important. We wish to show that when a small amount of DNA is added to the RNA, the synthesis of product is enhanced greatly over that expected from the sum of the two separately primed reactions. We shall also describe experiments dealing with the state of purity of the enzyme.

Regarding the first point, we have found, using highly purified *E. coli* ribosomal RNA (<0.05% DNA), that the addition of about 0.05% DNA to the reaction system leads to a three to ten-fold increase in synthesis. The same effect is

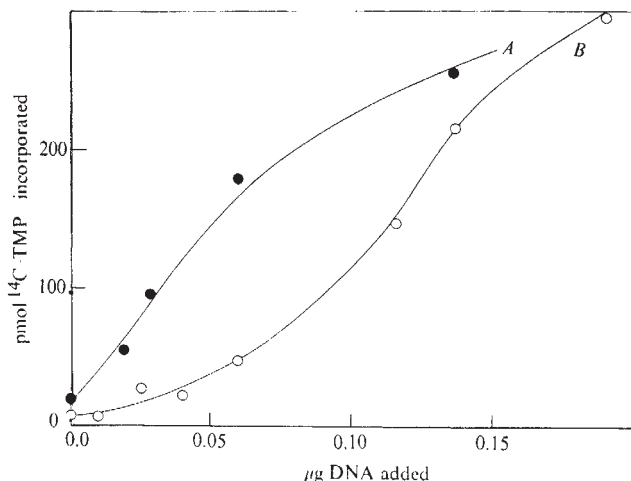


Fig. 1 Effect of added calf thymus DNA on the RNA-DNA polymerase reaction, curve A. The reaction mixture contained, in 0.5 ml., 25 µmol Tris, pH 7.8 at 25° C, 1 µmol each of magnesium chloride and β-mercaptoethanol, 10 µg of *E. coli* ribosomal RNA (16S+23S), 75 µg DNA polymerase (step 4)⁷ 4.5 nmol each of dATP, cCTP, dGTP and 7 nmol of ¹⁴C-TTP, specific activity, 17 mCi/mol (Schwarz Bio Research). The reaction mixture was incubated for 1 h at 37° C, chilled in ice-water and mixed with 0.1 ml. of 0.07% albumin followed by 1 ml. of 10% trichloroacetic acid. The mixture was washed on 'HA Millipore', dried on planchets and counted in an end-window counter. The acid precipitable radioactivity of an unincubated reaction mixture (<5%) was subtracted from each value. The number of pmoles incorporated was calculated from c.p.m. not correcting for counting efficiency (ca. 20%). The ribosomal RNA was prepared from *E. coli* B and had less than 0.05% DNA contamination. ●, (16S+23S)+DNA; ○, DNA only.

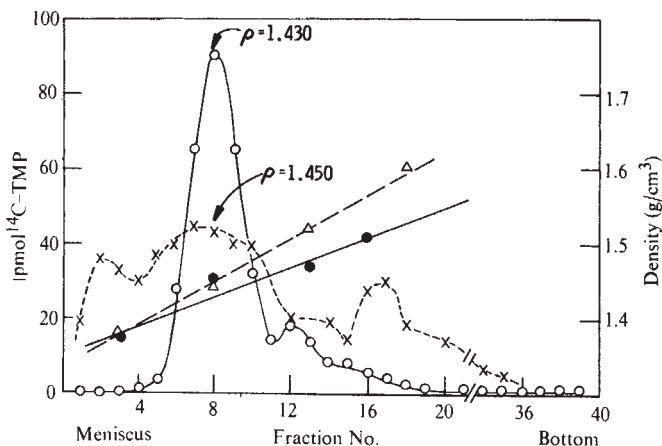


Fig. 2 Sedimentation equilibrium in a caesium sulphate gradient. The reactions were carried out as described in the legend to Fig. 1, except that the reactions were scaled up four times. After the reaction mixture was chilled in ice-water, it was dialysed for 2 days against 0.05 M Tris, pH 7.8, and then mixed with saturated caesium sulphate solution to a density of 1.550 g/cm³. Samples were centrifuged for 65 h at 32,000 r.p.m. at 25° C. Fractions were collected from the top and assayed as in the legend to Fig. 1. Densities were calculated from the refractive index measurements. ×, DNA enhanced reaction (3 h incubation), the corresponding density gradient is indicated by △. ○, The poly dA enhanced reaction (1 h incubation), the corresponding gradient is indicated by ●.

observed, but to a smaller extent, when poly rA·rU is used instead of RNA. These results are consistent with the interpretation that either DNA catalyses an RNA or poly rA·rU-instructed reaction or the latter two catalyse a DNA-instructed reaction.

A possible explanation might be the elimination of an inhibitor present in the partially purified DNA polymerase that was used⁷. There may be an inhibitor of DNA synthesis in the partially purified enzyme, as shown in Fig. 1, which shows that the response to added DNA is not initially linear with concentration. When the enzyme solution is aged for a day or two at 4° the inhibitor is inactivated, yielding a normal saturation curve corresponding to the extrapolation of the upper two points in the figure. The extent of synthesis, however, is still less than when the reaction is carried out in the presence of RNA.

The upper curve in Fig. 1 shows DNA synthesis with fresh enzyme (that is, containing the putative inhibitor) in the presence of RNA. The points of this curve have been repeated a number of times and are often much higher than shown; the variability depends in part at least on the age of the enzyme solution. Because the RNA concentration is constant, the point at zero DNA concentration indicates the increment expected all along the curve for the RNA-primed reaction. DNA and RNA are clearly synergistic in the reaction, although it is difficult to interpret the basis for the observation.

The purified DNA polymerase⁸ shows a normal type curve for synthesis *versus* DNA concentration. But we cannot conclude that the purification procedure removes the inhibitor since the entire procedure takes about 2 days, long enough to allow substantial spontaneous inactivation of the inhibitor. The addition of neither RNA nor poly rA·rU leads to enhancement of synthesis with DNA and the purified enzyme, although both are active separately. Some factor required for the RNA-DNA synergism also appears to be either unstable or eliminated during purification.

The requirements of the RNA-DNA reaction indicate that a heteropolymer is formed, for the omission of any one of the triphosphate substrates suppresses synthesis almost completely. Sucrose density gradient sedimentation velocity analysis of a 1 h product reveals that the product is relatively large but heterogeneous. There is a broad peak at about 4.2S and one at 8–10S. Sedimentation equilibrium in a caesium sulphate gradient shows a broad peak centred at a density of 1.446. The 3 h product has a major peak at a density of 1.450, together

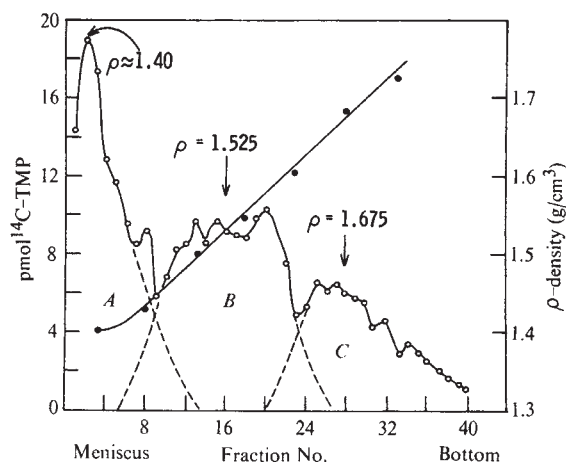


Fig. 3 Sedimentation equilibrium in a caesium sulphate gradient of the product obtained after 3 h of incubation (Table 1) using RNA alone. Analysed as described in the legend to Fig. 1.

with less dense and more dense peaks (Fig. 2). It seems clear that one of the products is rich in dA plus T ($\rho = 1.43$); the product in the densest region (ρ about 1.6) probably contains RNA.

The nature of the product(s) changes with the length of incubation, as shown in Table 1. Here the base composition of the acid precipitable material is given as a function of time for reactions primed by RNA alone, DNA alone and mixture of RNA and DNA (w/w, 10/0.058). With the RNA-DNA system, there is enhancement of synthesis over the single systems, as noted above. All reaction systems, however, show a very large increase in dA plus T in the product after 3 h of synthesis. This implies that a second reaction is occurring, perhaps the synthesis of poly dAT.

Table 1 Base Composition as Function of Time in the RNA-DNA Reaction

Systems	Incubation (h)	Incorporation (pmol)			
		dAMP	TMP	dCMP	dGMP
16S+23S RNA; no DNA	1	12.9	12.0	20.6	20.0
16S+23S RNA; no DNA	3	725.0	1,310.0	7.4	10.2
No RNA; DNA	1	21.4	14.7	19.4	15.4
No RNA; DNA	3	160.0	262.0	22.8	8.2
16S+23S RNA; DNA	1	110.0	104.0	85.0	100.0
16S+23S RNA; DNA	3	1,070.0	2,030.0	51.0	58.0
None; none	3	6.6	29.0	4.0	2.3

The reaction was carried out and processed as described in the legend to Fig. 1. In each case 7nmol of radioactive substrate (specific activity 17 mCi/mol) was used. Where indicated, 10 μ g of *E. coli* ribosomal RNA and 0.058 μ g of calf thymus DNA were used.

The product obtained after 3 h of incubation of a reaction mixture containing only ribosomal RNA as the polynucleotide (Table 1) was banded in a caesium sulphate gradient (Fig. 3). There are at least three major products (indicated as areas A, B and C) with densities of about 1.40, 1.525 and 1.675. The first is that to be expected from a polynucleotide rich in dA and T. Area B probably represents an RNA-DNA hybrid, the presence of which was demonstrated previously by its resistance to RNAase⁶. The highest density corresponds to RNA; we conclude from this that some TMP is bound to the RNA, which was unlabelled, either covalently or by hydrogen bonds.

To see whether or not the RNA-DNA system is unique, we examined the effect of replacing DNA by synthetic homopolymers. Poly dA plus RNA produces marked synthesis of a product containing dA plus T which is probably the homopolymer poly dA-T, as both substrates are required for the reaction to occur. In the absence of RNA, very little (3%) dAMP is incorporated. The incorporation of dCMP and

dGMP in this reaction is the same as with RNA alone. The homopolymers poly dG and poly T are relatively inactive both in the presence and absence of RNA. In the case of poly dC, there is considerable incorporation of dCMP and dGMP, which is not enhanced when RNA is present. For poly dA-T the incorporation is increased three-fold when RNA is present; this system, therefore, is similar to the RNA-DNA systems. The presence of RNA had no effect on the poly rA-T reaction; indeed, poly rA-T alone is the most active polymer discovered to date. In the poly dA-stimulated reaction, the omission of TTP as a substrate leads to virtual cessation of synthesis; omission of either dCTP or dGTP has little effect. The molecular weight is high as shown by the sedimentation constant (=14). Sedimentation equilibrium in a caesium sulphate gradient indicated a density of 1.430 for most of the material, but a minor peak at about 1.46 is always present. The latter peak may be the same as those obtained in the RNA reaction and the RNA-DNA reaction.

In conclusion, we have demonstrated a striking increase in DNA synthesis when traces of DNA are added to the *E. coli* enzyme system and RNA or poly rA-rU. The probable existence of an unstable inhibitor of DNA synthesis in the enzyme preparation has also been demonstrated, but this is insufficient to account for the synergism between RNA and DNA. Although the synergism is difficult to interpret, it indicates the need for caution in studying the template roles of RNA and DNA in systems such as some of the oncogenic viruses, which contain both types of polynucleotide^{9,10}.

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Interactive Computer Display for the Three Dimensional Study of Macromolecular Structures

THE application of real-time, three dimensional computer graphics to molecular structure studies has been limited so far to rather expensive, dedicated computer display devices^{1,2} or to fairly simple structures. The frequently used display device has been a black-and-white cathode ray tube³ with a split image or rotating image. This communication discusses the application of a colour television monitor to macromolecular modelling. Even with a small, 4,096 word laboratory computer driving the graphics display, it is now possible to view three dimensional models of large portions of macromolecular structures determined by X-ray diffraction studies.

Fig. 1 Stereo pair of a graphical representation of sperm whale myoglobin. All atoms drawn are within 15.0 Å of the iron atom. Large circles represent oxygen atoms, small circles represent nitrogen atoms. Figs. 1 and 2 were photographed from a black-and-white television monitor using program Display⁷. It should be noted that the prime output device is the colour monitor. Separate black-and-white prints are used here for ease in reproduction, and, although they illustrate the interactive zoom feature, they are not as emphatically three dimensional as images produced by the colour television monitor itself.



I am now assembling a library of protein structures for three dimensional display in conjunction with the Department of Chemistry, Brookhaven National Laboratory, for educational and research purposes.

Recently, several less expensive, three dimensional computer graphics display devices have become commercially available. One such device, incorporated into the Brookhaven Raster Display^{4,5} (BRAD), has been used to create an interactive display of molecular structures using a generalized set of Fortran computer programs. The BRAD system is part of the Data Terminal Network⁶ (DTN) developed at Brookhaven National Laboratory.

A three dimensional graphical image is created on a colour television monitor^{4,7} by drawing the stereo pair in different colours and by viewing the coloured images through coloured filters so that each eye sees only one colour (one of the stereo pair) but the mind of the viewer merges the two views to produce the effect of depth. (A limited number of illustrative prints and glasses is available.) The two stereo views correspond to two dimensional projections of the object rotated $\pm 3^\circ$. The images are created in digital form by a computer as they would appear superimposed on a 512 by 512 grid. Chemical bonds are indicated by lines between atoms. Circles or symbols may be used to designate specific atoms. The digital information is written on a peripheral memory (disk). The rotation of the disk and the data transfer rate are synchronized with the raster scan speed of a colour television monitor; the digital information is converted to video signals to produce a flicker free image. The colours red and green are used in the BRAD system described above.

In order to use the BRAD system for molecular and structural studies, a series⁷ of computer programs has been written to accept atomic coordinates, generate a connectivity table, create a library of a number of structures, retrieve a desired structure with a teletype command, add symmetry operations, rotate the display, and form the stereo images for display on the colour television monitor. These programs have been written in Fortran IV to accept a variety of structures and can, for example, process the structural library of the Crystal Data Centre at the University Chemical Laboratory, Cambridge, England, to create an information retrieval system^{8,9}.

Because of a recent increase in the DTN user memory (to 20.4 kilowords), the maximum number of atoms in the crystallographic asymmetric unit that may be operated on by the above set of programs has been increased to 400. Although the programs are capable of handling most small, crystallo-

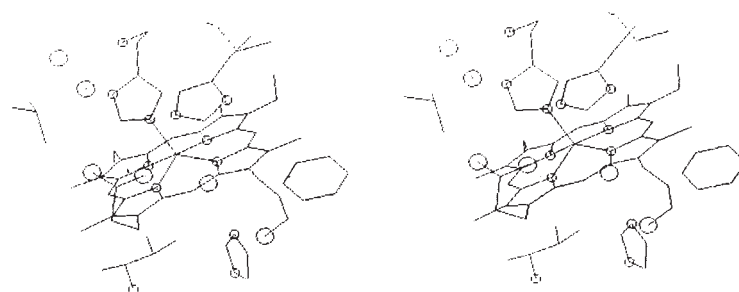
graphically determined structures, 400 atoms is only a fraction of the total number of atoms in a protein. Several data handling routines have been written, therefore, to operate on the basic set of atomic coordinates of a given macromolecule and extract the desired atoms for introduction into the above series of programs.

One such program can extract the coordinates of all atoms within a specified radius of a specified atom. For example, with the myoglobin¹⁰ structure, coordinates of all atoms within 10 Å of the iron atom can be extracted for viewing. The generation of symmetry-related atoms and unit cell translations for the study of intermolecular interactions is an optional feature of this program. A number of such segments, together with the usual display of the α -carbon backbone, for example, can be generated to form a small library for a particular protein. All the programs are written in a general form so that any available set of coordinates may be treated automatically.

Several new features that utilize the interactive, real-time aspects of the computer display have been added to the operational program package. The track-ball pointer is used to designate specific atoms and then to initiate several options. The track-ball is an analogue to digital (x - y) device that moves a spot of light one raster point in dimension about the face of the television screen. When "misses" or the overlap of atoms occur, the operator may try again. With one or more "hits", options to zoom in on a particular portion of the display or extract molecular geometric information may be called. Using myoglobin as an example, the operator may point at the iron atom in the haem group. After receiving the radius in Ångstroms, the program redraws the display (Figs. 1 and 2), scaling lines and circles so that the desired volume element is displayed. The view from different directions may be obtained by varying Eulerian rotation angles. Then, to explore the coordination of the iron atom, the track-ball pointer can be used to point at the iron and neighbouring nitrogen and oxygen atoms. Output consists of bond distances, bond angles, and torsion angles. Alternatively, the distance of n atoms from the least squares plane passing through the first m atoms may be obtained by means of the track-ball pointer. In this way, the geometry of the active site of an enzyme may be studied to the precision of the input coordinates.

Another set of routines permits incorporation and manipulation of molecular subgroups. In a generalized form, a vector between atoms a and b may be defined. Atom b and all atoms connected to it may be translated and rotated inde-

Fig. 2 The same as Fig. 1 except that only those atoms within 7.5 Å of the iron atom are included. This illustrates the interactive zoom feature.



pendently of the other atoms. The incorporation of a model of a substrate into a model of the active site may be performed under operator control with this interactive feature.

There are several implications of such a three dimensional display system. Most apparent is the fact that even the most progressive laboratory will have difficulty building and storing scaled models of macromolecules as they continue to become known. Applications to biochemical studies are being developed. Operator controls of the display which is being installed at Texas A and M University are being designed so that an individual or a group of students may study various molecular structures with a minimum of instruction.

The research implications to the biochemist include the ability to manipulate the peptide backbone or individual amino-acid residues to consider orientations available to the molecule in the liquid state. We have mentioned the possibilities of studying intermolecular interactions in the solid state and the capability to study the geometry of micromolecular-macromolecular interactions. The nature of the display permits similar structures to be merged or superimposed so that it may be useful in studying the relationship of structure and function of macromolecules. Crystallographically, the display is capable of model building and model manipulation in conjunction with programs to calculate structure factors, and so on.

A system under development in this laboratory (supplied by Syntex Analytical Instruments, Inc., with initial graphics routines written by Dr Robert Sparks) has a Data General Nova computer (4,096 words) to drive the display disk. A moderately complex structure is drawn in approximately 30 s. Such delays preclude real-time rotation, which is nonessential as the three dimensional effect is obtained from the coloured images.

Because of steady decreases in component costs, colour television displays like the above system, coupled to a mini computer, can be expected to become generally available. This should be of special interest to the structural biochemist who needs easier modelling techniques to keep up with the progress in macromolecular crystallography. Textbooks and demonstration methods now involve an unnecessary delay. A generalized set of computer programs such as those described above is capable of adding a new protein structure to the library with several man hours of preliminary preparation. As continual improvements are made, the preparation time should become minimal.

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Thermoluminescence of Biological Materials

THERMOLUMINESCENCE (TL) of fossil bones and of various kinds of recent biological material has been reported by Jasińska and Niewiadomski¹, who suggest that such materials could be used for dating purposes, but draw attention to difficulties which arise due to tribothermoluminescence (TTL, which is thermoluminescence derived from the mechanical energy of grinding) and to chemiluminescence (CL) associated with residual organic material.

A very satisfactory spatially uniform TL of artificially irradiated modern bone was observed in this department in 1966 and samples of fossil teeth and bones from Vértesözlös in Hungary were supplied by the late Dr Vertes in 1967 from one of the earliest camp sites of *Homo sapiens* in Europe, perhaps 50,000 yr old.

For the past 2 yr we have been working on the elimination of the two problems independently discovered by Jasińska and Niewiadomski and, although we have not yet eliminated entirely the irrelevant sources of light, we have reduced them sufficiently to make an approximate dating of the older and therefore more heavily irradiated materials. Much of the CL is due to simple oxidation and can be avoided by the standard procedure of heating the material in nitrogen during readout. Most of that remaining can be eliminated as follows. The bone, dental enamel or other material is first crushed to a coarse powder. This is then extracted for a few hours in a series of changes of 80% aqueous ethylene diamine in a Soxhlet extractor² which removes more than 99% of the organic matter and reduces the CL to a level below that of the TTL. The TTL is reduced by crushing the material to a powder suitable for measurement after the organic extraction. The material is then much more friable and needs less expenditure of energy. Because it is derived from friction, TTL is a surface effect and is further reduced by rejection of the finer powder which, having a large surface to volume ratio, gives a greater ratio of TTL to TL.

Fig. 1 shows the effects of CL, TTL and recovery on the shape of the "glow curves" from fresh bone powder. Curve N

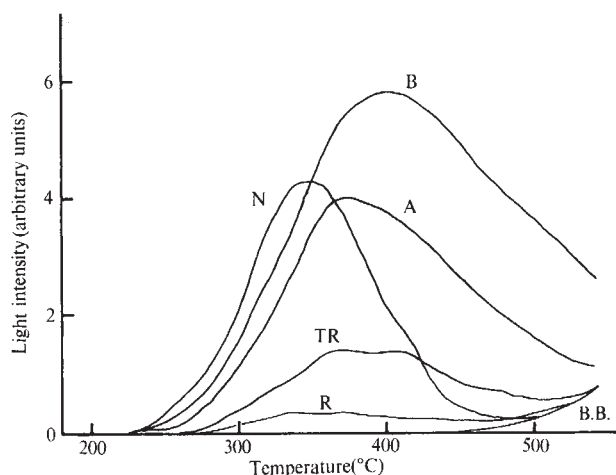


Fig. 1 Glow curves from differently treated samples of unirradiated fresh bone from the same source.

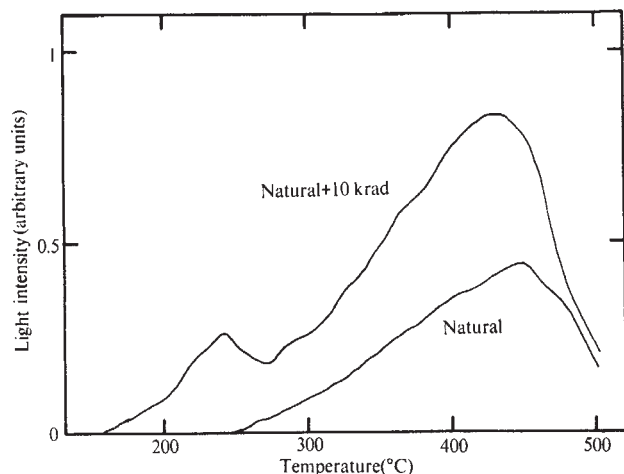


Fig. 2 Natural and artificial glow curves of an iron age bone cleaned by ethylene diamine for 17 h.

is the glow curve of untreated powder with grain diameters between 150 and 355 μm . Above 300° C the powder turns from white to black. Curve A shows the glow from bone powder of the same grain sizes but cleaned with ethylene diamine for 18 h. A decrease in CL is seen below 350° C. An apparent increase is seen above this temperature but, during the heating of untreated powder (N), the resulting blackening of the grains makes light collection very inefficient, which is why the light intensity drops above 350° C. TTL is seen by comparing curve A with B which was obtained from the same cleaned powder but had grain diameters below 45 μm . Powder already heated for curve A was recrushed and reheated, resulting in glow curve TR which demonstrates further the importance of TTL. This powder was then left on the heating strip for 30 min. On reheating, glow curve R was obtained. This recovery of TL remains unexplained, but it is possible that the contraction of the powder after the last heating causes the observed TL by a mechanism similar to that responsible for TTL. The amount of black body radiation is shown (curve B.B.).

The combined effects of residual CL and TTL are equivalent to a radiation dose of the order of 10 krad which is still too great for good results to be obtained in the region of 10–100 thousand yr. For example, Fig. 2 shows the “glow curves” of an iron age bone after it was cleaned by ethylene diamine. The glow area between 300 and 460° C varies with dose as shown in Fig. 3. By extrapolation, the natural radiation dose absorbed by the bone since the iron age is found to be approximately 15 krad, which would indicate an age some thousands of years higher than expected. Useful measurements could be made

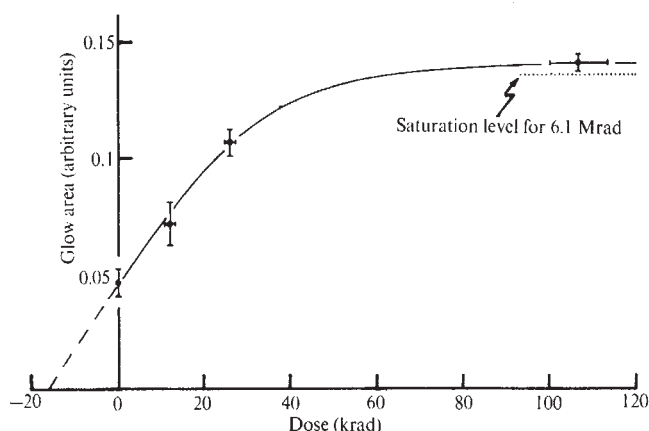


Fig. 3 Variation with γ -dose of the glow area between 300 and 460° C for the iron age bone of Fig. 2.

between 100,000 and a few million years. Above ten million years or so, saturation effects will progressively reduce the attainable accuracy.

Further research on the elimination of CL has concentrated on the use of enzymes such as collagenase and pancreatic enzymes. Limited success has been achieved, with reductions of CL by up to 50%, and more systematic work is in progress for the determination of the optimum temperature, pH and duration of the extraction.

Several problems remain other than the further reduction of these irrelevant glows. These include determination of the relative contributions to the total natural irradiation by (a) the internal radioactivity of the samples themselves (usually small), (b) the surrounding material in which they have lain, and (c) cosmic rays. This may be laborious but is not difficult. Also needed is an estimation of the extent to which chemical change or accretion of thermoluminescent material (for example, calcium carbonate) during storage may affect the results. This estimation may vary from very easy to quite impossible and will involve careful selection of material. The internal enamel of the complex molar teeth of herbivores and the shells of some molluscs seem to be the most promising of the materials which we have tried so far. The removal of organic matter was found to be unnecessary for dental enamel from a 2–3 million year old rhinoceros tooth and samples of this nature seem to present little difficulty to TL dating workers³.

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Cannabis Alkaloids

SCORES of studies scrutinizing psychotropic phenolic terpenoids (cannabinol derivatives) of *Cannabis sativa* L. (Moraceae) have diverted attention from the possible presence of other potentially active classes of compounds, such as alkaloids, in marijuana. Recently, simple nitrogenous zwitterionic compounds (muscarine, choline and trigonelline) as well as volatile, low molecular weight piperidines have been isolated from several related species including hemp^{1–4}. But more complex compounds of the alkaloid type have not been reported.

Alkaloidal mixtures were extracted from plant material by freezing and grinding it to a fine powder in liquid nitrogen and distributing between methylene chloride and dilute aqueous acid and alkali several times. When this method was applied to confiscated commercial Mexican *C. sativa* leaves, stems and flowering tops (brick marijuana, referred to hereafter as dried material), we isolated alkaloidal material (soluble in organic solvents and aqueous acid, insoluble in aqueous alkali) in 0.02% total crude yield. Tests with classical alkaloid detection reagents (potassium mercuric iodide, potassium iodoplatinate) were positive.

Greenhouse cultivation of thirty-four of these dioecious plants from viable seeds in the confiscated marijuana proceeded quite satisfactorily⁵. The average plant sprouted in 12 days, flowered in 100, and attained a height of 250 cm when harvested for extraction at age approximately 200 days. Of the total aerial weight of the typical plant, 42% was leaves. Pollination of female pistils was done by hand from the male plants. Comparative studies revealed that alkaloid was concentrated

Table 1 High Resolution Mass Spectrometry* of Cannabis Alkaloids

Alkaloid	Cannabamine A	Cannabamine B	Cannabamine C	Cannabamine D
Volatilization temperature (°C)	128	125	128	170
Probable molecular ion	363.2882, 363.2904	299.1509	279.1591	311.2553
Probable molecular formula	C ₂₁ H ₃₇ N ₃ O ₂ , C ₂₆ H ₃₇ N	C ₁₈ H ₂₁ NO ₃	C ₁₄ H ₂₁ N ₃ O ₃	C ₁₇ H ₃₃ N ₃ O ₂
Theoretical value for M ⁺	363.2876, 363.2916	299.1521	279.1583	311.2573
Unsaturation No.†	5, 4	9	6	3
Base peak	43	299.1509	86.0970	240.1703
Probable base peak formula			C ₅ H ₁₂ N	C ₁₂ H ₂₂ N ₃ O ₂
Theoretical value for boiling point			86.0969	240.1712

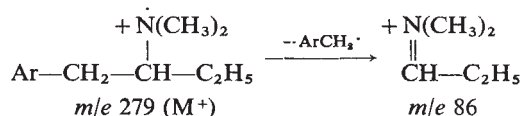
* Consolidated Electrodynamics Corporation 21-110b spectrometer (70 eV) was used.

† Number of double bonds and/or rings in the molecule.

in the leaves of the plant rather than the stems or roots; yields of purified total crude alkaloid from fresh leaves averaged 0.003% and male and female plants contained equal amounts of alkaloid. The presence of three alkaloids was established by silica gel thin-layer chromatography using ethanol containing 2.5% diethylamine as eluent. For convenience we have named these compounds cannabamine A, B and C, in order of decreasing polarity. Cannabamines A, B and C were also present in dried marijuana along with a fourth, least polar, alkaloid, cannabamine D. Cannabamine A was the most abundant and cannabamine B the second most abundant alkaloid in both fresh and dried *C. sativa*.

Since the alkaloid mixtures from fresh and dried *C. sativa* were practically identical, subsequent experiments were carried out on alkaloid isolated from the more convenient latter source. Preparative thin-layer chromatography of the total alkaloid furnished pure samples of the four cannabamines for mass spectrometry. Using the method of direct insertion into the mass spectrometer ion source, the temperature of each sample was gradually raised over several hours; a mass spectrum of each volatile effluent was taken, and high resolution measurements were made of the ostensible molecular ion and the base peak. All spectra thus obtained were quite definitive and had relatively few peaks contributing significantly to the total ionization of the alkaloid.

High resolution mass spectrometric data, summarized in Table 1, indicate medium to high molecular weight oxygenated alkaloids. Although it is impossible to formulate conclusive structural assignments from these data alone, in the case of cannabamine C the abundant C₅H₁₂N⁺ fragment suggests a β-arylethylamine alkaloid which, on electron impact, readily cleaves to give a stabilized benzylic radical (ArCH₂·) and the observed base peak, *m/e* 86.



Loss of a C₅H₁₁· radical from the molecular ion of cannabamine D is of potential biogenetic interest in view of the occurrence of normal amyl groups in cannabis terpenoids.

Total crude alkaloid from dried marijuana dissolved in 0.1 N hydrochloric acid and adjusted to pH 4 with aqueous sodium hydroxide was used for pharmacological testing. Quantitative thin-layer chromatographic analysis of the total crude alkaloid for psychotropic cannabinoids⁶ indicated that such compounds, if present at all, constituted less than 0.3% of the mixture of cannabamines A, B, C and D used in testing.

Three different batches of extract (each at pH 4.0, about 0.5% total crude alkaloid) were tested for pharmacological activity and toxicity in mice. Extract 1 was given to seven mice in doses of 0.1–0.5 ml. subcutaneously, intraperitoneally and intravenously. There were no gross signs of toxicity and all seven animals were alive 24 h later. Three mice were injected with 0.5 ml. subcutaneously and placed in an activity cage⁷ which registered minimal motor activity for 60 min and slight activity from 60 to 120 min. Control mice injected

with 0.5 ml. normal saline solution (pH 4.0) showed decreased motor activity in the same cage for less than 5 min. Similar activity studies were done with extracts 2 and 3. Three mice given 0.15, 0.4 and 0.4 ml. of extract 2 subcutaneously showed minimal activity for 90 min and three mice given 0.5 ml. of extract 3 subcutaneously showed decreased activity from 15 to 130 min after injection. None of the mice used in the activity studies showed any signs of drug action other than decreased activity. A portion of each extract was used to determine possible effects on pentobarbital sleeping time in mice and 0.4 ml. subcutaneously given 20–30 min before pentobarbital administration did not affect sleeping time after either 45 or 80 mg/kg sodium pentobarbital.

Thus, cannabamines A, B, C and D represent the first alkaloidal compounds with pharmacological activity characterized from *C. sativa*. Even though only small concentrations of these compounds are present in the plants their significance cannot be dismissed in light of the possibility of cumulative effects of the chronic use of cannabis preparations. Research on Cannabis alkaloids is continuing in these laboratories; full experimental details will be reported in a future publication.

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Sex Pheromone Mimics of the American Cockroach

DURING an investigation of several essential oil preparations for juvenile hormone activity¹, we discovered that a volatile component(s) in several coniferyl needle distillates stimulated apparent sexual excitement in male American cockroaches,

Periplaneta americana (L.). The response, consisting of movement, wing flutter, extended abdomen and attempted copulation, was behaviourally identical to that elicited by the natural sex pheromone of this insect² and in marked contrast to the behaviour (hyperactivity) induced by exposure to highly irritating chemicals such as amyl acetate.

Needle and cone oil distillates of spruce, *Picea rubra* Link, and fir, *Abies siberica* Ledeb., *Abies alba* Mill., were fractionated by column chromatography over 'Florisol', and the active fractions were examined by gas-liquid chromatography. (A column 4 mm x 2 m was used, packed with 0.75% methyl silicone on 200 mesh gas chrom P.) The effluent of the column was directed into a 2 l. jar containing ten male cockroaches which had been isolated from female cockroaches for 6 weeks. Sexual display by the cockroaches was associated with only one peak in these fractions. Final purification of the pheromonal substance was carried out by preparative gas chromatography. (An F and M Model 775 Prepmaster equipped with gold plated injector block and stainless steel columns 0.75 inches x 16 feet was used. It contained 4% methyl silicone on 150 mesh 'Chromosorb W'.)

By its pleasing and characteristic odour the active compound was tentatively characterized as bornyl acetate, a known constituent of the source trees. Comparison with standards by gas-liquid chromatography and by infrared, nuclear magnetic resonance and mass spectroscopy confirmed its identity.

Bioassays were performed according to Bodenstein³. Thus, the compound in hexane solution, was absorbed on a piece of Whatman No. 1 filter paper (1 cm²) and held within the 2 l. rearing jar with forceps. The sensitivity of male cockroaches to the natural sex pheromone and to the pheromone mimics is greatly magnified by maintaining the males in isolation from females for at least 4-6 weeks before attempting bioassays. The response of newly segregated males is erratic or indifferent except to extremely high concentrations of natural pheromone, whereas isolation for several weeks makes them highly sensitive and uniform in response.

Related compounds such as camphor and borneol were inactive, although the propionate ester prepared from borneol was very active. Other plant-derived essential oils were examined for activity and, of these, α - and β -santalol were found to be equivalent to bornyl acetate in activity with *Periplaneta* (Table 1). Although the L and D bornyl acetate standards were of the highest optical purity obtainable it is possible that the slight activity of the L isomer was due to minute contamination with the D isomer. Alternatively, partial racemization may take place during the period of exposure or at the receptor site of the insect.

After these studies we examined several flowering plant species for sex pheromone-like activity with *Periplaneta* by briefly soaking various parts of the plants in hexane or methanol and offering the extract on filter paper to the isolated groups of male cockroaches by the usual bioassay. Of one hundred common plants, eighteen were found to produce behaviour characteristic of exposure to the natural sex pheromone (Table 2). The freshly cut stems of several species, notably *Aralia spinosa* L. and several Compositae produced strong

Table 2 Flowering Plants³ containing Hydrocarbons causing Sex Pheromone-like Behaviour in *Periplaneta americana* Male Cockroaches

Simaroubaceae
<i>Ailanthus altissima</i> (Mill.) Swingle
Araliaceae
<i>Aralia hispida</i> Vent., <i>nudicaulis</i> L., <i>racemosa</i> L., <i>spinosa</i> L.
Labiatae
<i>Perilla frutescens</i> (L.) Britton
Compositae
<i>Achillea millefolium</i> L.
<i>Ambrosia artemisiifolia</i> L., <i>trifida</i> L.
<i>Chrysanthemum cinerariaefolium</i> (Trev.) Vis., <i>leucanthemum</i> L., <i>morifolium</i> (Ramat.), Hemsl.
<i>Erigeron annuus</i> (L.) Pers., <i>canadensis</i> L.
<i>Eupatorium hyssopifolium</i> L., <i>purpureum</i> L.
<i>Rudbeckia hirta</i> L.
<i>Solidago juncea</i> Ait.

responses from the male cockroaches without solvent extraction. The active material seemed to be present mostly in the fruits and in the stems just beneath the outer cuticle, and varied with the season or stage of growth of the plants. In addition to these plants a very active hydrocarbon fraction has been obtained from the fruits and buds of *Liquidambar styraciflua* L. and *Liriodendron tulipifera* L. The pheromonal principle was readily obtained by rinsing the plants (or steeping for a few minutes) in hexane. Column chromatography over Florisol gave a highly active hydrocarbon fraction. Final purification was achieved by column chromatography on silver nitrate coated silica gel. The active hydrocarbon constituent from several of these plants was found to be identical in a comparison by gas-liquid chromatography, infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy. In the mass spectrum, the parent ion gave a molecular weight of 204, consistent with the empirical formula C₁₅H₂₄. This assignment was fully supported by the fragmentation pattern. Approximately 0.1 μ g of this material was capable of exciting eight out of ten groups of isolated male cockroaches. A study of its structure has been undertaken.

The discovery of a diversity of structures which elicit this characteristic sexual display in *Periplaneta* is very disconcerting in view of the oft reported specificity of sex pheromones. Although a relationship between these plant products and the natural cockroach sex pheromone may eventually be established, we are certain that none are identical with the natural attractant. To investigate this possibility we extracted virgin female midguts as described by Bodenstein³, obtained a highly active lipid extract. The active principle withstood refluxing in 5% methanolic potassium hydroxide but was destroyed by treatment with 10% methanolic base. Pheromonal activity was destroyed by brief treatment with sodium borohydride in methanol and then quantitatively regenerated by oxidation with chromic acid in acetone. Treatment of the crude extract in hexane solution with a drop of bromine eliminated activity, but debromination with zinc dust in 5% acetic acid in ether restored about one-half the original activity. This brief study leads us to speculate that the natural attractant is an unsaturated ketone with a hindered ester moiety or containing other base labile groups. By trapping the effluent from gas chromatographic columns we have obtained unweighable samples (< 50 μ g) of the natural pheromone which can be diluted 1 billion times and still elicit the characteristic mating display from male cockroaches. Thus, none of the plant derived pheromonal mimics are as active as the natural pheromone.

We hope that the information derived from structural characterization of the active plant hydrocarbons taken together with the known active compounds such as bornyl acetate and santalol will provide leads in the elucidation of the structure of the natural sex pheromone.

Although it has been shown that a plant substance is necessary for sex pheromone release in an insect⁴, this is the first report of a sex pheromone-like substance taken directly from plants.

Table 1 Compounds that Elicit Sexual Display in Isolated Male *Periplaneta americana*

Compound	Active concentration* (mg/cm ²)
L-Bornyl acetate	7.0
D-Bornyl acetate	0.07
α -Santalol	0.07
β -Santalol	0.07
C ₁₅ H ₂₄ plant hydrocarbon	0.14

* Threshold concentration of compound on 1 cm² of Whatman No. 1 filter paper which induces sexual display in at least eight of ten groups of male cockroaches containing ten males per group.

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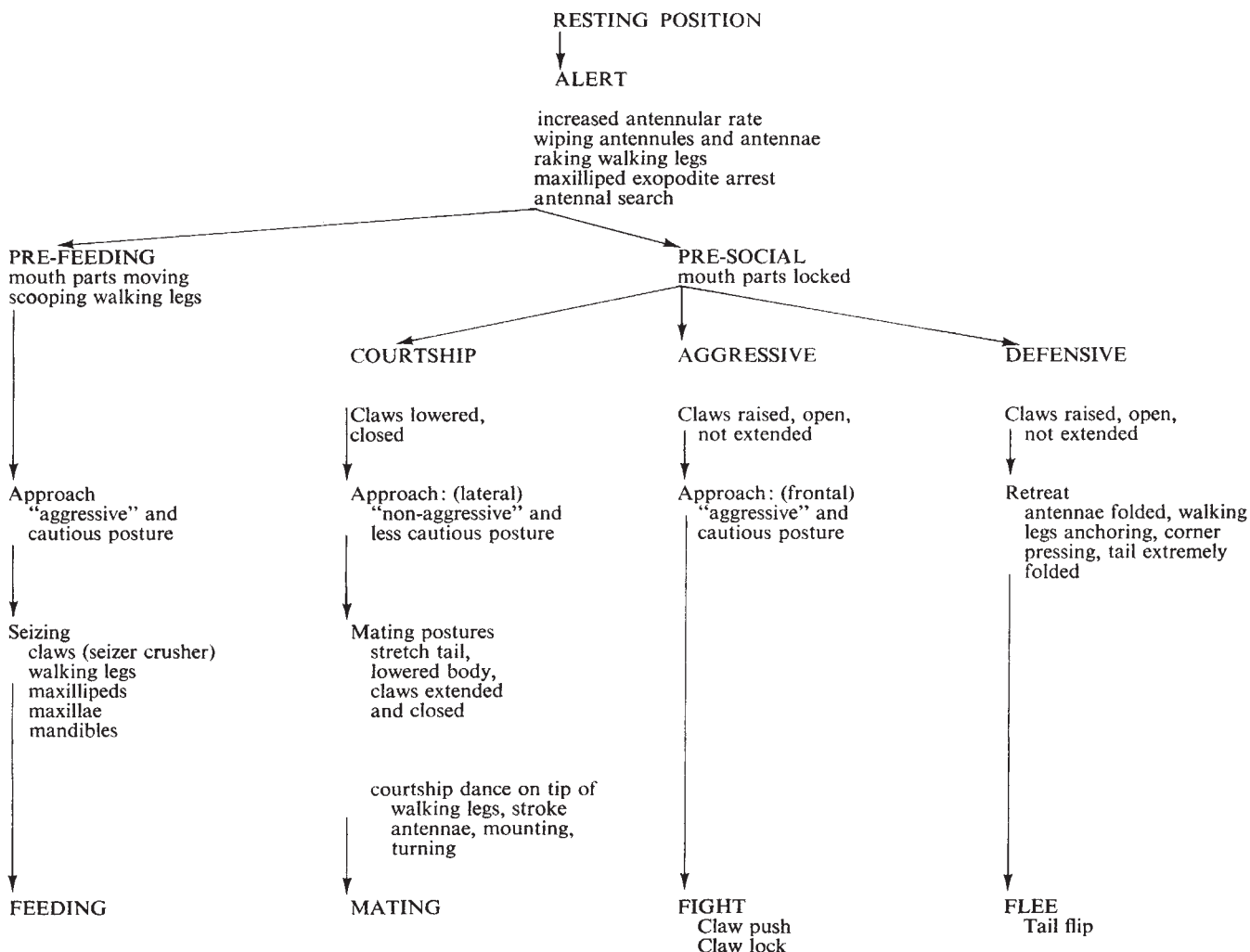
Sex Pheromone in the Lobster, *Homarus americanus*

SEXUAL recognition and attraction in many insects have been shown to involve chemical communicants called pheromones¹, and these results are widely applied in agriculture². In contrast

very little is known about (sex) pheromones in crustaceans, the only experimental verification being that of Ryan³ who observed that males of the Pacific crab, *Portunus sanguinolentus*, displayed towards water from premolt females. No response was obtained after the excretory pores of the females had been sealed. A few other publications on crustacean behaviour and natural history suggest such a chemical communication system. Berry⁴ observed in *Panulirus homarus* that "male lobsters show sudden attraction to particular sexually mature females" and speculated that the tegumentary glands may be involved. Hughes and Matthiessen⁵ stated that "it appears as if the freshly moulted female (*Homarus americanus*) exerts a chemical attraction on the male". Some other indications for the existence of a sex pheromone can be found⁶ without experimental evidence, however. This lack of experimental evidence, even in reports on crustacean mating behaviour⁷, is remarkable considering the economic importance of such crustacea as lobsters and shrimp and the benefits for culturing these animals, if a sex pheromone were known and available.

Sexually mature lobsters were trapped in Woods Hole in the late spring of 1970, averaging 78 mm in carapace length with little variation in size. They were kept individually in 50 l. fibre glass tanks with 20 cm of water, and a flow of 1 l. min⁻¹ from the seawater supply of the National Marine Fisheries Service (formerly the Bureau of Commercial Fisheries) in Woods Hole. Some lobsters were kept in plexiglass aquaria to allow photographic recording from all sides, and all were on a

Table 1 Behaviour Units and Sequences



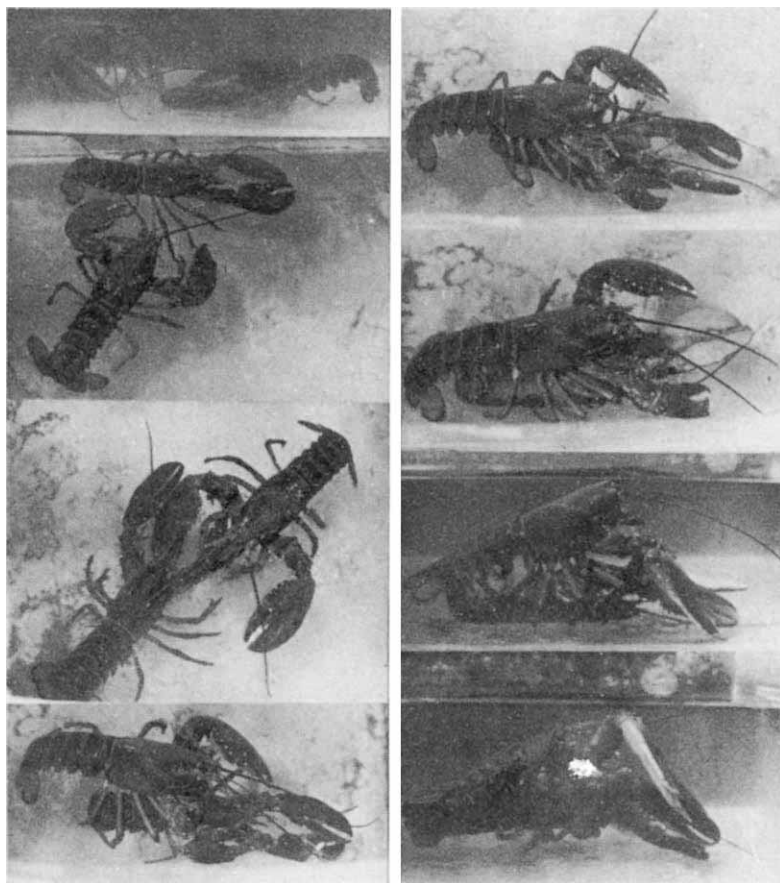


Fig. 1 Mating in *Homarus americanus*.

natural day–night schedule and were fed twice a week on fish and molluscs.

Two types of experiments were carried out: (a) interactions in which two lobsters were brought together and their social behaviour recorded, and (b) water tests, in which the water of the tank of one animal was introduced into the tank of another, whose responses were recorded. The animals were tested in different combinations of sexes and moult stages.

Two trained observers recorded the behaviour using a verbal code, and in many cases on film using a Beaulieu 8004 Z camera and artificial lights. (A 25 min film was also made and shown at the film session of the Animal Behaviour Society at the AAS meeting in Chicago, December 1970.) Analysis of the recordings enabled us to describe behaviour in well defined units. The most important units of social and feeding behaviour were categorized (Table 1), providing clear criteria for distinguishing pertinent units and sequences.

Our experiments showed that non-moulted (or hard shell) lobsters fight when brought together. Frontal approaches and pushing with the claws are followed by locking the crusher claws in a wrestling match of pushes and pulls. The dominant animal then chases the subordinate who flees and assumes submissive postures. Bodily damage may occur at this stage. Fights were observed in male–male and male–female interactions. Female–female experiments were not done.

After a non-moulted male lobster has been introduced into the tank of a female moult (a moult is a newly moulted lobster) his behaviour is distinctly different from the very beginning (Fig. 1). He seems to be immediately aware of the presence of a sexually ready female. His claws are lowered and closed and he brings himself up on the tips of his walking legs. He approaches the soft shelled female very slowly and gently, walking around her and stroking her continuously with his antennae. On several occasions this behaviour which was devoid of any aggression appeased an initially unwilling female. After about 15 min of this courtship dance, the male slowly mounts the female from behind and turns her over with his walking legs. Copulation takes about 8 s, after which the two

animals separate and find a corner position in the tank. The male is still not aggressive towards the vulnerable female. In a few cases, moulted females attempted to initiate copulation in unwilling males by making slow lateral approaches for about 15 min, but the males were still not aggressive.

In the water tests, we presented male and female animals with water from the tanks of naturally occurring moults, induced moults through bilateral eyestalk clipping and moults that had occurred elsewhere and been sent to us. We used water from non-moulted males and females as controls. To collect the water for testing, the flow to the donor tank was shut off for 4–6 h. It was necessary to hold induced moults for 8–13 h before the tests. Observations were made for 30 min, during which 2 l. of test water was siphoned into the tank at 200 ml. min⁻¹. The behavioural criteria (Table 1) were again used to analyse the responses toward the different chemical communicants, including the water containing sex pheromone and its chemically isolated fractions. These experiments showed that water from the tanks of non-moulted males and females failed to elicit responses from either males or females. The behavioural responses of male and female test animals towards water from the tanks of the different groups of moulted lobsters are summarized in Table 2. The responses elicited by male moult water are contrasted with those elicited by female moult water and act as additional controls.

The female lobsters noticed the introduction of water from the tanks of moulted male and female lobsters, but were not attracted to it in the observation time of 30 min. Male lobsters, on the other hand, were attracted to water from both males and females. Their behaviour showed aggressive and feeding patterns after water from a moulted male had been introduced, but never after that from a moulted female. In the latter case the males displayed behaviour units characteristic of the sexual repertoire. The responses of males to moulted male and female water corresponded in each case to the behaviour recorded when these animals were brought together in interactions.

As well as these experiments, we siphoned the pheromone into a tank containing a non-moulted (not emitting) female and then

Table 2 Results of Water Tests

Water from	Priming time (h)	Test animals	Alert	Search	Touch tube	Comments on behaviour
(No. responses/No. tests)						
♂ Normal moult (1) (in labs)	4-6	Male	2/2	2/2	2/2	Aggressive
		Female	2/2	0/2	0/2	Alert only
♀ Normal moult (2) (in labs)	4-6	Male	7/7	7/7	2/7	Courtship, non-aggressive, not pre-fed
		Female	1/2	0/2	0/2	Alert only
♀ Normal moult (1) (im-ported)	4-6	Male	4/4	4/4	4/4	Courtship, non-aggressive, not pre-fed
		Female	1/1	0/1	0/1	Alert only
♀ Induced moult (2)	8-13	Male	3/3	3/3	3/3	Low intensity responses
		Female	2/2	0/2	0/2	Alert only

introduced a male. The resulting interaction was unique and unsuspected. The animals were aggressive for about 6 min, pushing and pulling with locked claws. After they had unlocked, the male started to court the female and even mounted her several times. This behaviour lasted about 8 min, after which the animals moved away from each other. The male remained unaggressive. But two males brought together in comparable circumstances were still aggressive and fought each other. We concluded that a chemical compound (sex pheromone) is present in the water of a newly moulted female lobster that suppresses aggression and induces courtship.

The biological activity survived boiling for 5 min in N₂. Isolation of the pheromone is in progress.

It seems that the lobster, as might be predicted from its anatomy, lives in a world where chemical communication is important. Our studies indicate that the reproductive behaviour is largely under pheromonal control. Induced moults (Table 2) produce less active water than normal moults, because they require a longer priming time and elicit low intensity responses. In interactions, males displayed aggressively and actually damaged induced-moult females. Not only do such females seem to produce less sex pheromone than naturally moulted females, they may even secrete a stress product or transmit an abnormal hormonal balance to the external milieu, as removal of the eye stalks also removes the most important endocrine glands⁸.

Although the division between smell and taste in Crustacea is poorly understood, behavioural analogies with insects¹ and even catfishes⁹ suggest strongly that social communication is linked with the sense of smell, and feeding behaviour with the sense of taste. In general, we might expect to find this functional distinction in the chemical senses of aquatic animals that depend on them heavily. A clear understanding of the total chemical environment with all its different chemical messengers for food, friends and foes as well as for health, sickness and population density is required in many aspects of aquaculture, in which crowded culture areas put further stress on the already abnormal water chemistry. The sex pheromone is only one of the many social communicants that we expect in the lobster.

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Inhibition of Nematocyst Discharge correlated with Feeding in a Sea Anemone, *Calliactis tricolor* (Leseur)

IN spite of much work¹ neither the mechanism nor the nature of control of the discharge of nematocysts is well understood. These stinging capsules, characteristic of coelenterates, have generally been assumed to be independent effectors^{2,3}, their discharge depending solely on external stimuli, with the animal exerting no control. Some coelenterates, however, seem to be able to modify nematocyst discharge⁴⁻⁸, and we feel that nematocysts should therefore not be considered true independent effectors. Working with the Florida sea anemone, *Calliactis tricolor*, we have observed a sharp decrease in the number of nematocysts discharged with increased feeding.

C. tricolor was collected on shells of the gastropod *Polinices duplicatus* inhabited by the hermit crab *Pagurus pollicaris* in the North Florida Gulf coast area. Specimens were removed from the shells and placed on glass dishes or empty *Polinices* shells in a 20 gallon recirculating sea water aquarium. They were fed *Artemia* nauplii regularly, but not for 24 h before experiments.

Previously nematocyst discharge has been quantified by counting the tentacles which adhered to a presented object^{4,6}, but we have devised a reliable, quantitative technique for measuring nematocyst discharge directly. Number two glass coverslips were coated with an aqueous extract of *Artemia* nauplii (prepared by grinding freshly hatched brine shrimp in a glass homogenizer and centrifuging to remove particulate matter) and air dried. When a coverslip was presented to the anemone, the tentacles adhered, leaving outlines consisting of many adhering discharged, and very few undischarged, nematocysts. The number of tentacles contacting the coverslip was recorded as a further indication of responsiveness. After staining the coverslip with 1% methylene blue, discharged nematocysts were counted in the three most dense fields along three separate tentacle outlines (traces) under $\times 440$ magnification (field = 0.094 mm²). Tests before and after staining revealed no measurable loss of discharged nematocysts from the coverslip or discharge of undischarged nematocysts due to the procedure. Areas of greatest density were counted instead of random counts because of uneven distribution of traces on the coverslip and of nematocysts within traces. Independent counts for a single coverslip gave consistent results. The average of three counts was used to calculate the index of discharged nematocysts for that coverslip, using the formula:

$$I = D \times A/B$$

where *I* is the Index of discharge; *D* is the average density of nematocyst discharge for three fields; *A* is the number of tentacle outlines on coverslip, and *B* is the number of tentacles

contacting coverslip. The factor A/B was equal to 1 initially, but at satiation often was as low as 1/5 or zero.

In each experiment three anemones were transferred from the aquarium (still attached to dish or shell) to a bowl containing 1,500 ml. of sea water (continuously aerated). After a 15 min acclimatization to test conditions, all three anemones were tested for initial level of nematocyst discharge. No differences in nematocyst discharge were seen, whether the animal's pedal disk was attached to glass or shell. Two of the animals were fed pieces of fresh frozen mullet (thawed at room temperature), approximately 0.1 g, each. In the same bowl, the third anemone (control) did not receive food.

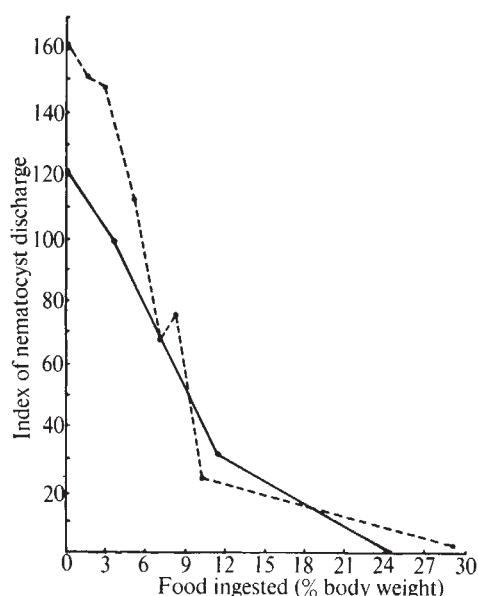


Fig. 1 Decrease in nematocyst discharge with increase in amount of food ingested (% anemone's body weight). ---, 6.84 g anemone; —, 3.28 g anemone.

The long, outstretched tentacles of a starved anemone contract rapidly after contacting a piece of mullet, bringing it quickly to the mouth. Within 30–60 s the tentacles regain their original length and may wave or twitch at their tips. This relatively vigorous response is given to the first several pieces. The response weakens with continued feeding; more time is spent in bringing food to the mouth, and the anemone frequently remains contracted for 1–4 min. The waving and length of the tentacles are reduced. Additional food remains on the tentacles several minutes before being brought to the mouth. At satiation, pieces of mullet are loosely held and eventually drop from the tentacles; no feeding reaction occurs. The control (unfed) anemone's appearance remains unchanged throughout and is indistinguishable from that of a starved anemone.

After being fed several pieces of mullet, all anemones were tested for nematocyst discharge. Feeding was resumed until no food would be ingested. When mullet pieces were dropped twice consecutively, the anemones were satiated and all were retested. Each trial ended at satiation. A significant decrease in index of nematocyst discharge was observed for all fed animals, whereas no significant decrease was observed for controls (Table 1).

Pieces of mullet were weighed and fed to two tared anemones, nematocyst discharge being tested after each piece had been ingested until satiation. Although one anemone weighed twice as much as the other, the amount of food needed for satiation was approximately the same percentage of each anemone's body weight (24.5%, 29%, Fig. 1).

To test whether nematocysts were being depleted by presentation of repeated food stimuli, food was carefully placed on one half of an anemone's tentacular crown (only these tentacles transported food to the mouth) while nematocyst discharge was tested on both halves and compared. No difference in the index of discharge at satiation between the two halves of the oral disk was observed. In experiments to ascertain the influence on nematocyst discharge of non-nutritive bulk in the gastro-vascular cavity, starved anemones ingested up to 1.75 times their weight in clean glass beads before reaching "satiation". Packing the gastro-vascular cavity with glass beads causes a noticeable decrease in nematocyst discharge, but the decrease is at most 60% of the decrease shown after satiation with food.

Table 1 Comparison of the Number of Nematocysts discharged to a Food Stimulus between Control (Unfed) and Experimental (Fed) *C. tricolor* Anemones

Experiment	Index of discharge Fed anemones		Control anemones	
	Start	Finish	Start	Finish
1	130 90	28 0	75	66
2	115 129	5 3	147	109
3	137 156	8 6	136	122
4	162 145	24 34	133	133
5	122 163	0 3	165	171
6	127 146	10 4	132	119
7	121 88	5 6	125	127
8	132 153	0 2	135	139
9	95 80	12 5	105	111
10	76 89	2 12	80	83
Range	76–163	0–34	75–165	66–171
Mean	118.8	8.4	123.3	118.0
Standard deviation (s_x)	9.52	2.13	9.01	9.20
t value	22.64 (highly significant)		0.82 (not significant)	

Twenty anemones were used; three per experiment—two experimental, and one control. The time for each experiment varied from 60–153 min (average 109 min). The number of pieces (about 0.1 g each) needed for satiation ranged from 7–29 (average 16).

Although the nematocyst has previously been considered an independent effector, these experiments indicate this may not be the case, at least for *Calliactis tricolor*.

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In vitro Cytocidal Effect of L-Glutaminase on Leukaemic Lymphocytes

MOUSE lymphomas, such as 6C3HED, undergo complete regression after one or more injections of L-asparaginase¹, and remissions have been reported in some patients with acute leukaemia after treatment with L-asparaginase²⁻⁴. Roberts *et al.*⁵ found that purified bacterial L-glutaminase inhibited the growth of Ehrlich mouse carcinoma. Before considering this enzyme for therapeutic studies in man, it is necessary to study *in vitro* effects of the enzyme on human blood cells. Previous work⁶ demonstrated that the cells of 6C3HED lymphoma do not survive incubation with very low concentrations of L-asparaginase (1.7 mIU/ml.), while the cells of the *in vivo* resistant variant of 6C3HED were not killed by 170 mIU of enzyme/ml. The blood lymphocytes from patients with chronic lymphocytic leukaemia were found to be more sensitive than normal lymphocytes to incubation with 170 mIU of L-asparaginase/ml. but not as sensitive as 6C3HED mouse lymphoma which responds *in vivo* to the enzyme. In this study, the slide chamber method⁷ was used to compare the sensitivity of normal and leukaemic lymphocytes to glutaminase.

The blood cells tested were obtained from five persons with normal haemograms, from eight patients with chronic lymphocytic leukaemia, and from three patients with acute leukaemia. Suspensions of the blood cells plus varying amounts of L-glutaminase were incubated at 37° C for 7 days. The suspensions were then examined in a special slide chamber and the surviving cells were counted. The criteria of viability depended on the morphological integrity of the cells under phase microscopy⁷.

The L-glutaminase was derived from bacteria and was described as preparation GA : 1.2 in a previous report⁵. The preparation also had L-asparaginase activity equal to 80% of the glutaminase activity. In all experiments, control tests were done with equivalent amounts of purified L-asparaginase to evaluate toxic reactions due to L-asparaginase.

A summary of the findings is given in Table 1. Most of the normal lymphocytes survived incubation with 1,700 mIU of glutaminase/ml. The cells of one patient (M.U.) with chronic lymphocytic leukaemia had approximately the same resistance to the enzyme as normal lymphocytes. In the case of the other seven patients with chronic lymphocytic leukaemia and of three

patients with acute leukaemia, very few cells survived incubation with 5 to 0.5 mIU of enzyme/ml.

The media of the incubated cells were analysed to determine the effect of the enzyme on the concentration of glutamine, ammonia and glutamate. As would be expected, the enzyme caused a decrease in glutamine (to <0.02 mmol) in suspension of both the leukaemic and normal lymphocytes. The ammonia and glutamate increased correspondingly to 1.0 and 0.8 mmol, respectively. In further experiments, the addition of 1 mmol of NH₄Cl or 0.8 mmol of sodium glutamate or both had no effect on the survival of leukaemic lymphocytes. These findings suggest that the decrease of glutamine in the media killed leukaemic but not normal lymphocytes *in vitro*.

According to our findings, the leukaemic lymphocytes from seven of eight patients were killed directly or indirectly by L-glutaminase, usually at the level of 1.7 mIU/ml. It is noteworthy that this dose of L-asparaginase was cytotoxic to cells of 6C3HED mouse lymphoma⁶ which undergoes remission *in vivo* with L-asparaginase therapy.

Normal lymphocytes resisted the concentration of 1,700 mIU of L-glutaminase/ml. although leukaemic lymphocytes usually responded to 1.7 mIU/ml. Previous work from this laboratory⁸ showed that the leukaemic lymphocytes are also more sensitive *in vitro* than normal cells to other reagents including L-asparaginase, prednisolone, dimethyl sulphoxide and heat. For example, leukaemic lymphocytes were sensitive to prednisolone at the level of 1 µg/ml. and to L-asparaginase at 170 mIU/ml. Glutaminase, however, acted at a much lower concentration than the other reagents and showed a much greater difference in its effect on normal and leukaemic cells.

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Table 1 Percentage of Lymphocytes or Blasts that survived Incubation at 37° C for 7 Days with Varying Amounts of L-Glutaminase

Source of cells	Cells surviving (%)							
	0	1,700	170	17	5	1.7	0.5	0.17
Normal persons (5)	53	33	35					
Patients with chronic lymphocytic leukaemia								
M. U.	32	18	11					
P. K.	46	—	3	3	3	13	33	59
K. O.	48	—	—	2	1	2	1	38
S. T.	39	—	—	0	0	1	5	29
C. S.	43	—	0	1	0	2	—	—
K. R.	76	—	0	4	1	3	—	—
H. U.	42	—	0	0	0	0	—	—
L. A.	65	—	4	8	11	7	43	66
Patients with acute leukaemia*								
P. H.	55	—	0	—	0	1	—	—
T. K.†	45	—	3	12	35	34	—	—
W. M.†	27	—	2	2	1	2	10	—

* P. H. had acute plasma cell leukaemia; T. K. had acute myelomonocytic leukaemia; W. M. had acute lymphoblastic leukaemia.
† Cells were incubated for 5 days.

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Inequality of Inspired and Expired Gaseous Nitrogen in Man

IN studies of animal respiration, it is conventional to assume that the amounts of nitrogen inspired and expired are equal, and this assumption has passed from one textbook to another¹⁻¹². The argument is that there is no known metabolic reaction of the human body involving molecular nitrogen, and

that in ordinary circumstances nitrogen is merely a diluent of the oxygen in the air breathed¹³. Three recent studies¹⁴⁻¹⁶ cast doubt on this assumption and have prompted us to use a direct system to detect changes in the amounts of inspired and expired nitrogen gas.

This investigation revealed an obvious but little known biphasic history of the theory of nitrogen involvement. The initial hypothesis, based largely on the works of Lavoisier¹⁷⁻²⁰, was that nitrogen gas was neither produced nor absorbed. There followed a period in which the consensus supported a change in the amount of nitrogen, although it was debated whether this change was positive or negative—production or absorption. Current thought has reverted to Lavoisier's original dictate that the amount of nitrogen expired is equal to the amount inspired. In neither of these sources, however, are direct experimental data presented, nor are earlier sources cited where such data are available.

Allen and Pepys determined^{21,22} that: (a) no change occurs in atmospheric air in respiration except the substitution of carbonic acid for an equal amount of oxygen; but (b) when pure oxygen is respired a portion of it is replaced by an equal amount of azote (N₂). In small animals, Edwards^{12,23,24} found that whether nitrogen was exhaled or absorbed depended on the season of the year (temperature) and the age of the animal.

In 1839 Boussingault^{25,26} measured the nitrogen in the food of a milch cow, a horse and a pigeon, and the nitrogen excreted by these animals. The output was less than the intake: 27 g/day for the cow, 24 g/day for the horse, and 1.14 g/day for the pigeon. He concluded that gaseous nitrogen is not assimilated in the process of respiration. Regnault and Reiset (1849)²⁷ studied small mammals, birds, amphibians and worms. Warm blooded animals exhaled nitrogen in from 1/100 to 1/50 the proportion of oxygen consumed. Animals in a state of inanition and occasionally cold blooded animals were found to absorb nitrogen. Barral^{27,28} used the same indirect method as Boussingault. In five humans he found the ingested nitrogen to be greater than the nitrogen in urine and faeces. The excess was said to be passed off by the lungs and skin. Berthollet *et al.*²⁹, Dulong²⁷, Berthollet and Nysten²³, Jurine and Nysten, Nysten and Treviranus²⁹ also observed an exhalation of azote (nitrogen).

This evidence notwithstanding, the exhalation of nitrogen was far from definite. Priestley²³ supposed that the gas was absorbed, and he was supported by Davy, Cervier, Henderson, Pfaff, Spallanzani, Humboldt and Provencal²³, and Marchand^{10,29}.

After this period of lively research there was a marked lack of interest in the subject, continuing until the current idea of non-change came to the fore. The link that seems to be missing is experimental evidence to support this shift and lack of interest. If this is true, then to attribute some of the experimental evidence for absorption or exhalation to error³⁰ or accident^{23,31}, while accepting Lavoisier's verdict without direct confirmation, appears to be a questionable solution to the problem.

Criticisms of Lavoisier's viewpoint and the validity of the classical technique for the determination of nitrogen balance (after Lusk)²⁶, have been made by Costa, Ullrich, Kantor and Holland¹⁵. The criticisms are based on evidence substantiating nitrogen loss in sweat and positive nitrogen balances in various mammals that failed to demonstrate a commensurate body weight gain in proportion with the nitrogen retention.

Several reports^{14,15,32} reveal interesting inconsistencies with the historic or classic concept of nitrogen non-involvement in respiratory gas exchange, and suggest that molecular nitrogen is an unrecognized metabolite and that quantities of nitrogen sufficient to balance the nitrogen equation could conceivably escape undetected among the expired respiratory gases. Costa *et al.* showed that some of the nitrogen fed is eliminated through this neglected pathway^{14,15}. With a closed system technique in which a nitrogen-free atmosphere was made with

helium-oxygen washout, a substantial increase in the concentration of nitrogen was measured with a mass spectrometer. The absolute amounts of elemental nitrogen produced in mice were approximately 14–28 mg/kg/24 h. These quantities are sufficient (5–10% of intake) to balance the nitrogen equation. In humans the estimated total production of nitrogen was in the range 8–24 mg/kg/24 h.

Levitt³³ used a constant perfusion technique and reported that hydrogen occurs in the expired air of humans. Of all the hydrogen produced in the gastrointestinal tract, about 14% appears in the expired air³³. Mitchell³⁴ concedes that nitrogen gas excretion via the lungs may occur in certain herbivores due to nitrogen liberating bacteria. Robin *et al.*³⁵ have found ammonia in the expired air of dogs. In non-steady state conditions, as when the barometric pressure changes, or when the temperature of the body fluctuates, gaseous N₂ may be dissolved or released from solutions in the body, and so appear as exhaled or retained nitrogen.

With this confusing background in mind we investigated whether or not gaseous nitrogen was being expired in excess of that inspired in humans, both at rest and during moderate exercise, always in steady state conditions.

All samples were collected by a syringe-metallized bag technique as described by Johnson *et al.*³⁶. Gas analysis was done with a Beckman paramagnetic oxygen analyser (model C2, sensitivity 0.15 mm Hg partial pressure of oxygen, O coefficient of variation) and a Godart NV carbon dioxide thermal analyser (model A2, gain 0.2, sensitivity 0.07 mm Hg partial pressure of CO₂, 1% coefficient of variation). N₂ was calculated by difference:

$$F_{N_2} = 1 - F_{O_2} - F_{CO_2} \quad (1)$$

The other calculations were made from Haldane's equations⁸.

At rest the subject was seated between two Tissot gasometers. Through a standard double Douglas valve he inspired from one gasometer and expired into the other. The room was kept at 24° C, 40% relative humidity. The system was calibrated by forcing random volumes of air from the inspired Tissot to the expired and analysing samples of each. During the treadmill walk the inspired air flowed from a Tissot gasometer of 800 l. capacity through a sampling chamber and then a Parkinson-Cowan dry gasometer and finally a sampling chamber. The subject breathed through a low resistance-high velocity Otis-McKerrow valve. Room temperature was controlled at 15.5° C, with a 50% relative humidity. The system was calibrated by forcing air through at rates like those of a man walking. Samples were collected and analysed as described here.

The results of thirty-six resting experiments corrected for calibration differences (Table 1) showed no average nitrogen production, but rather a mean negative figure of -27 ml./min which would indicate absorption. There were nine positive, twenty-seven negative and no equal results. This was significant to the 0.01 level by a χ^2 analysis. The historical literature survey had also demonstrated production or absorption under different conditions, so it was then our supposition that if any nitrogen had been produced it might be accentuated in walking conditions and thus be consistently measurable with our equipment. This proved to be the case. In the thirty-five walking experiments thirty gave positive results, that is, expired nitrogen was greater than inspired. On the basis of χ^2 analysis this result was significant at the 0.001 level. The mean difference was 132 ml./min at a pulmonary ventilation of about 16 l./min. The mean N₂ excess was about 2 ml./kg min (Fig. 1).

Our results are similar to those of Costa *et al.*^{14,15}, though our values are rather higher. Our experiments also show wide inter and intra-individual variations, for which we have no explanation. The demonstration of molecular hydrogen in man³³, with 14% of the total hydrogen production of the intestines excreted by the lungs, suggests that some mechanism may exist for diffusing nitrogen. In any case, there do seem

to be grounds for questioning the doctrine of equality for inspired and expired nitrogen.

One important practical question raised by this apparent production of nitrogen in man is, What effect does it have on respiratory calculations? How much difference does it make? We have taken one of the most extreme cases for resting and one for walking and made a comparison between the actual measured values and values obtained by the Haldane calculations (Table 2). In our conditions the carbon dioxide produced was closely approximated either by actual measurement or by Haldane's assumption. In these two extreme cases, however, the correct oxygen consumed in fact was as much as 12% lower than that calculated from the expired air alone by Haldane's method.

Table 1 Inspired and Expired Nitrogen in Humans Sitting Resting

A Subject and test*	B Expired N ₂ l./min †	C Inspired N ₂ l./min †	D (B - C) l./min
S. P. 1	5.715 4.046 3.734	5.781 4.033 3.533	-0.066 +0.013 +0.201
L. D. 1	6.128 6.015 6.025	6.156 6.012 6.101	-0.028 +0.003 -0.076
H. I. 1	5.723 5.672 6.201	5.830 5.756 6.210	-0.107 -0.084 -0.009
J. P. 1	4.004 3.239 3.190	3.959 3.232 3.251	+0.045 +0.007 -0.061
S. P. 2	4.436 4.540 4.727	4.414 4.562 4.726	+0.022 -0.022 +0.001
L. D. 2	5.663 5.347 5.005	5.704 5.432 5.052	-0.041 -0.085 -0.047
H. I. 2	5.929 5.455 5.549	5.938 5.566 5.604	-0.009 -0.111 -0.055
J. P. 2	3.762 3.665 3.528	3.855 3.716 3.617	-0.093 -0.051 -0.089
S. P. 3	4.727 4.561 4.025	4.409 4.593 4.048	+0.318 -0.032 -0.023
L. D. 3	5.902 5.684 6.134	5.935 5.800 6.280	-0.033 -0.116 -0.146
H. I. 3	5.575 5.868 5.953	5.658 5.915 5.959	-0.083 -0.047 -0.006
J. P. 3	4.616 4.727 4.541	4.678 4.779 4.502	-0.062 -0.052 +0.041
N	36	36	36
Mean	4.989	5.016	-0.027

* Tests were about 1 week apart for any given subject.

† Corrected for differences in calibration between intake and output Tissot gasometers.

Table 2 Actual and Calculated Values for Oxygen consumed and Carbon Dioxide produced when Expired Nitrogen is greater than Inspired Nitrogen

A Item measured or calculated (l.)	B Resting (J. P.) (a) Actual value*	(b) Haldane calculation	C Walking (L. D.) (a) Actual value*	(b) Haldane calculation
(a) Air inspired	5.573		19.312	
(b) Air expired	5.673	5.673	19.342	19.342
(c) N ₂ inspired	4.404	(4.534)†	15.301	(15.702)†
(d) N ₂ expired	4.534	4.534	15.702	15.702
(e) N ₂ produced	0.130	(0)†	0.401	(0)†
(f) CO ₂ produced	0.237	0.240	0.801	0.801
(g) O ₂ consumed	0.266	0.302	0.970	1.060

* Extreme cases in these studies. All volumes are reduced to STPD.

† Assumed in the Haldane calculation (equation (1)).

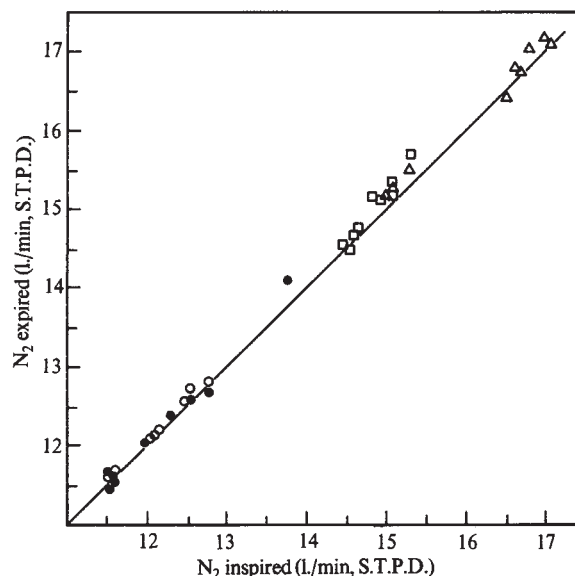


Fig. 1 Gaseous nitrogen inhaled and exhaled during walking on the level at 6 km/h. Four subjects were used, three men: L. D. (□); H. I. (△); J. P. (●), and one woman: S. P. (○). Values are in l./min STPD including calibration corrections. The line $y=x$ is for the case when the two values are equal, as assumed in the Haldane transformation. In actual experimentation, however, in thirty of thirty-five cases the nitrogen expired was greater than the nitrogen inspired. By χ^2 analysis, the probability of this difference being random is less than 0.001.

Our data suggest that the open circuit method is subject to hitherto disregarded sources of error. These may be accentuated in physical exercise. For the present argument, it is no matter whether the excess nitrogen is metabolic, gastrointestinal, bacteriological or dissolved in tissues—the effect on the calculations is the same. In some circumstances both the inspired and expired air may have to be measured directly.

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Patterns of Regeneration between Individual Nerve Cells in the Central Nervous System of the Leech

SEVERED AXONS of nerve cells can regrow towards their original destinations and re-establish synaptic connexions. For example, optic nerve fibres in the frog and goldfish grow back to the appropriate regions of the midbrain^{1,2}, and motor nerve cells in crayfish reinnervate their original muscles³. The new connexions seem to be correct, in the sense that normal function is restored by regenerating fibres growing back to the region that had been denervated. But it is not known how precisely an individual nerve cell can find its original target. One possibility is that each cell accurately reforms all its original synapses. On the other hand, the mechanism might be less precise, and the result still be functionally adequate or better, even though some cells have become reconnected abnormally. The general question of what happens at the cellular level during regeneration could also be relevant for an understanding of how specific neural connexions are formed during development.

In the experiments reported here, we have used the relatively simple central nervous system of the leech to study the regeneration of connexions between single, identified nerve cells, rather than large populations of neurones. The segmental ganglia in this animal are all similar, each containing only about 350 nerve cells. Earlier work has shown that fourteen sensory nerve cells can be identified reliably in ganglia from any animal⁴. Each of the cells has a characteristic set of properties by which it can be recognized, including (a) its shape and position in the ganglion, (b) the electrical properties of its

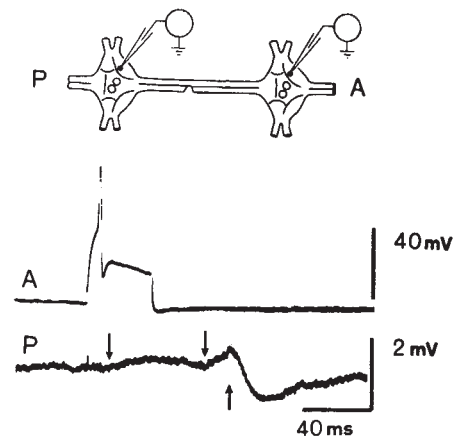


Fig. 1 Intracellular recordings from touch cells in adjacent ganglia of a normal leech. The arrangement for stimulating and recording is shown above. A single impulse in the touch cell in the anterior ganglion (A, upper trace) gives rise to two depolarizing synaptic potentials and a hyperpolarizing potential, indicated by arrows in the posterior touch cell (P, lower trace). Similar potentials were seen in the anterior cell following an impulse in the posterior cell. The cut in the lower connective disconnected the touch cells on that side of the two ganglia, so that stimulation of either cell no longer evoked synaptic potentials in the other.

membrane, (c) the specific type of stimulus to which it responds (touch, pressure or noxious mechanical stimuli), (d) the position of its receptive field and (e) the synaptic connexions that it makes with other cells in the same ganglion and in adjacent ganglia. The principle of the experiment is to cut one of the bundles of axons (a connective) that links two segmental ganglia, and see whether certain of these sensory cells become reconnected.

In normal preparations which have not been operated on, the interconnexions of these sensory cells are specific and stereotyped⁵. Thus, the three touch cells on one side of one ganglion are connected to their homologues on the same side of each adjacent ganglion by way of fibres running through the ipsilateral connectives (Fig. 1). One or more impulses in any one of these three cells gives rise to complex synaptic potentials in all three touch cells in both of the adjacent ganglia. These connexions are "specific", in the sense that impulses occurring in touch cells do not evoke synaptic potentials in sensory cells of different modality or in many other cells that we can identify and have recorded from in adjacent ganglia. The traces in the lower part of Fig. 1 show the synaptic potentials evoked in a touch cell (P) after a touch cell (A) in the ganglion immediately anterior to it was stimulated by passing current through the intracellular microelectrode. The post-synaptic responses consist of depolarizing potentials, followed, after a variable delay, by a hyperpolarizing potential. Similar responses occur in touch cells in adjacent ganglia with stimulation in the opposite direction, that is, from posterior to anterior. All these interactions are disrupted when one cuts the connective on the same side as the sensory nerve cells. We have not yet located the cell bodies of any interneurons involved in the interactions of touch cells. Stimulation of a connective evokes hyperpolarizing and depolarizing synaptic potentials in touch cells.

To study regeneration, leeches were anaesthetized with 8% alcohol, and the connectives between a pair of ganglia exposed under the dissecting microscope; no one particular region of the animal was selected. One of the two connectives was cut completely. To be sure that the cut had severed the whole of the connective, it was always extended across the midline, so as to nick the other connective, which served to hold the two ends in approximate apposition. The skin was sutured with fine thread. After the leech had recovered from anaesthesia, a constriction of the body wall appeared at the site of the lesion and the swimming movements were abnormal: the two halves of the animal above and below the lesion no longer acted in

concert. Within a few weeks, the animals once again appeared normal, but the recovery of function was not a reliable sign of regeneration, because many leeches swam normally even though the cut ends of the severed connective had not rejoined. We do not know what changes in the nervous system could account for this restoration of the normal behaviour.

In eleven leeches, studied 5–24 weeks after operation, there was clear evidence of regeneration of nerve fibres. At the site of the lesion the connective which had been severed earlier was restored almost to its original thickness. In the preparation shown in Fig. 2, the arrows indicate where the upper connective had been cut 40 days previously (see also Fig. 4, below). In all the experiments, just after the preparation had been dissected out of the animal for study, the other control connective was cut to prevent conduction in all but the regenerated fibres. Electrical recordings from such preparations showed that impulses were conducted through the chronic lesion in both directions. The arrangement for stimulating and recording from connectives with external electrodes is shown in the diagram of Fig. 3. Stimulation of the connective in this way also gave rise to impulses which propagated through the ganglia, as well as synaptic potentials in various cells by way of regenerated fibres that had grown through the lesion. Thus, both excitatory and inhibitory potentials were recorded in the touch cells, and depolarizing synaptic potentials were recorded in the "pain" cells, after stimulating the regenerated connective (Fig. 3). These experiments showed, therefore, that nerve fibres had regenerated and formed synaptic contacts in the ganglion beyond the lesion.

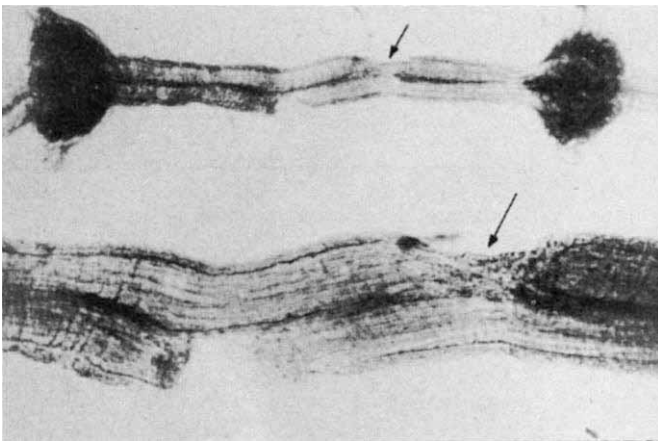


Fig. 2 Photographs of a preparation taken from a leech in which one connective had been severed completely 40 days earlier. The other connective was cut just before taking the photograph (lower connective in the figure). The site of the lesion in the regenerated connective is indicated by arrows. (The diameter of a connective is approximately 100 μ m.) This is the preparation from which the records shown in Fig. 4 were taken.

We next investigated the specificity of the newly formed synaptic connexions by stimulating and recording from individual nerve cells in adjacent ganglia with intracellular microelectrodes (see ref. 4 for the methods). In every preparation in which regeneration had occurred, some of the touch cells on the side of the lesion became reconnected: impulses in a touch cell in one ganglion gave rise to synaptic potentials in one or more of the touch cells on the same side of the next ganglion by way of the regenerated connective. An example is shown in Figs. 2 and 4. Here, one connective had been cut in the animal 40 days earlier, and had now rejoined. As usual, the other connective (below in Fig. 2) was cut just before the experiment, disconnecting the touch cells in the lower part of the two ganglia. On the side of the original lesion, a single impulse in an anterior touch cell then gave rise to a synaptic potential in a posterior touch cell and vice versa. The con-

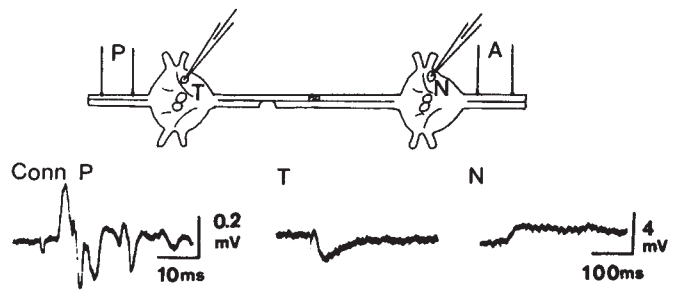


Fig. 3 Recordings made from a connective and from individual nerve cells in two preparations operated on 111 and 90 days earlier. In the diagram, A and P are external electrodes for recording from or stimulating the connectives. The normal connective is cut acutely just before the experiment. The compound action potentials recorded at P following stimulation of the anterior connective at A are shown on the left trace (Conn P). This preparation had been operated on 111 days earlier. The middle trace (T) shows the hyperpolarizing synaptic potential recorded from a touch cell in the anterior ganglion after stimulation of the posterior connective at P (preparation operated on 90 days beforehand). The right trace shows a depolarizing synaptic potential in a "pain" cell (N) in the anterior ganglion following stimulation of the anterior connective (same preparation as in trace Conn P, 111 days).

nexions between these two touch cells were, however, different from those in normal control animals in which both depolarizing and hyperpolarizing potentials were evoked in either cell after stimulation of the other (Fig. 2). Instead in the regenerated preparation of Fig. 4 an impulse in an anterior touch cell (A_1) gave rise only to a depolarizing, synaptic potential in a posterior cell (P_1), while an impulse in a posterior touch cell (P_2) gave rise to a hyperpolarizing potential in the anterior cell (A_2). A similar failure of the regenerated connexions to restore both types of synaptic potentials between touch cells has been found in all the other animals: the synaptic potential observed in the posterior touch cell was in the depolarizing direction, whereas that in the anterior touch cell was hyperpolarizing. In some instances the inhibitory potential was immediately preceded by a small depolarizing potential as in Fig. 4.

The connexions between touch cells in adjacent ganglia did not always regenerate in both directions. For example, in the experiment illustrated in Figs. 2 and 4, in one pair of touch cells (A_2 , P_2) a depolarizing or a hyperpolarizing synaptic potential was observed when the anterior or the posterior cell was stimulated; in another pair (A_1 , P_1) only stimulation of the anterior touch cell gave rise to a synaptic potential, and in the third pair stimulation of either cell was ineffective.

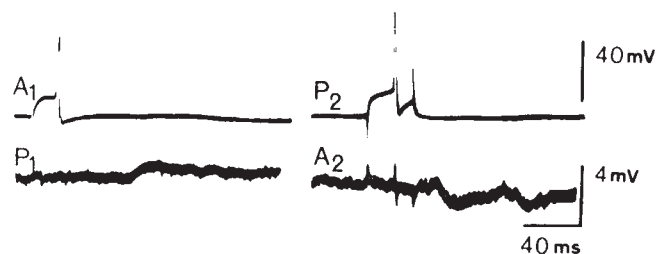


Fig. 4 Synaptic potentials in touch cells in adjacent ganglia in a preparation operated on 40 days previously (see also Fig. 2). The control connective was cut just before the experiment. An impulse in an anterior touch cell (A_1) gave rise to a depolarizing synaptic potential in a posterior touch cell (P_1). Stimulation in the opposite direction was ineffective in this pair of cells. The traces on the right show hyperpolarizing synaptic potentials evoked in a different touch cell in the anterior ganglion (A_2) by stimulation of another posterior touch cell (P_2). With this pair of cells stimulation worked in both directions: an impulse in A_2 gave rise to a depolarizing synaptic potential in the posterior touch cell P_2 (not illustrated). The third pair of touch cells were not connected in either direction.

The regenerated connexions can be considered to be "specific" rather than random, because impulses in a touch cell gave rise to synaptic potentials in touch cells, but not in any other sensory cells or in many other identified cells that we recorded from. Moreover, stimulation of these other types of cells did not cause potentials to appear in touch cells. All the interactions we have found so far have been between cells known to be connected in the normal animal.

The results therefore show that functional reconnections of certain individual neurones do occur in the regeneration process. We do not yet, however, have anatomical or physiological evidence to decide whether the same neurones find their old targets directly, by way of the same interneurons, or whether some other compensatory regrowth occurs. Neither can we say whether the regeneration we have seen is due to axons becoming joined up at the cut ends of the connective³ or to outgrowth from the proximal ends of the fibres followed by synapse formation in the ganglion. Nevertheless, whichever mechanism is correct, one would expect a high likelihood of random (that is, wrong) connexions, unless some form of directive mechanism had occurred. Several thousand axons run along each connective, while the neuropile contains the complex arborizations of all the 350 neurones in the ganglion, as well as the terminals of fibres coming from the connectives and peripheral nerve roots. This in turn implies that there must be some cellular recognition mechanism capable of distinguishing one particular neurone out of thousands of others.

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Use of Two Cerebral Hemispheres to increase Brain Capacity

STUDIES of split-brain cats and monkeys demonstrate that each cerebral hemisphere possesses its own perceiving, learning and remembering system, and that each functions independently of the other. Section of the mid-brain commissures also creates two independently functioning systems in the human brain¹. The split-brain monkey can process and respond to more information involving bimanual motor sequencing than can control animals with their commissures intact², and commissurotomy patients perform double voluntary reaction time tasks as fast as they carry out a single reaction³. In man, two verbal messages projected to separate hemispheres are dealt with more efficiently than two messages directed simultaneously to the same hemisphere⁴, and reaction times are shorter when the

information is projected separately to each cerebral hemisphere⁵.

Capacity can be assessed by asking an individual to perform two tasks at the same time⁶. The capacity remaining for the performance of the second task can provide a measure of the demands of the first task on the system. If in a situation of overload of this kind the signals of one task are projected in such a way that they are shared between the cerebral hemispheres this should lead to an increase in the total capacity of the brain which will be reflected in the amount of the task that the individual can perform.

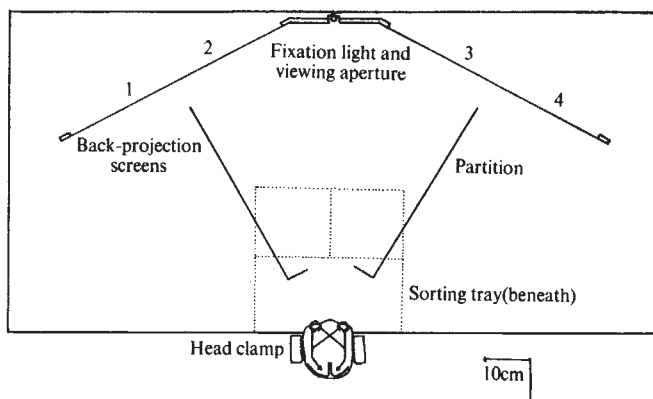


Fig. 1 Plan diagram of apparatus.

Each of twelve first year undergraduates was confronted by four display screens (28 × 44 cm) arranged with two partitions as in the plan shown as Fig. 1. Signals on the screens to the left of the subject are directed to the right hemisphere of the subject and signals on the screen to the right of the subject are directed to the left hemisphere. Signals on screens 1 and 4 are directed through nasal hemiretinae, and on screens 2 and 3 through temporal hemiretinae. Eye movements are monitored through the central aperture.

The subject was asked to report pairs of random black digits (30 cm high) arranged into blocks of eighty trials, in two of which (LVF blocks) the digits appeared on screens 1 and 2, in two (RVF blocks) on screens 3 and 4, and in two (BVF blocks) on screens 1 or 2 and 3 or 4. Screens within the BVF blocks and block orders were balanced. The rate of presentation throughout each block was an intertrial interval of 1 s and an exposure time of 250 ms. Simultaneously with this task the subject was required to sort nuts and bolts into two bins, using both hands, out of his sight below the apparatus, but conveniently within reach. Trials of sorting alone were given at the start and finish of the experiment.

The results were analysed by taking the mean of the two corresponding blocks for each subject (Table 1). On testing by means of a *t* test it is shown that for the visual task, LVF blocks are inferior to RVF blocks ($P < 0.001$) and to BVF blocks ($P < 0.001$). The difference between RVF and BVF blocks is significant at $P < 0.01$. Comparable results are found in the sorting task in that LVF and RVF blocks differ at $P < 0.05$, while sorting alone is significantly superior to all other conditions ($P < 0.001$). No other comparisons on the sorting task reach statistical significance.

In addition an analysis was carried out of the trials in the BVF condition to eliminate the possibility that part of the effect shown might be the result of superior performance in either one eye or one retinal hemifield. The results are given in Table 2, and it will be seen that while two comparisons do just reach acceptable levels of significance (LN+LT v. LT+RT, RN+RT v. LT+RT), the pattern of results cannot support any simple hypothesis that either one eye is superior to the other or that temporal and nasal retinal hemifields differ.

Table 1 Performance on Simultaneous Visual and Sorting Tasks

Visual task (pairs of digits correctly reported):			
	N	Mean	s.d.
LVF :	12	61.04	8.16
RVF :	12	69.29	5.99
BVF :	12	74.83	4.76
Sorting task (number of nuts and bolts sorted):			
	N	Mean	s.d.
with LVF blocks —correct :	12	70.25	19.13
errors :	12	3.00	2.45
with RVF blocks —correct :	12	76.75	13.35
errors :	12	2.79	2.12
with BVF blocks —correct :	12	75.83	18.52
errors :	12	2.20	2.08
sorting alone —correct :	12	98.67	17.23
errors :	12	1.54	2.39

LVF, Left visual field; RVF, right visual field; BVF, both visual fields.

Table 2 Performance of Different Hemispheres on the Visual Task

Visual task: BVF condition (correct responses in two blocks: maximum=40):			
Retinal hemifields to which stimuli presented	N	Mean	s.d.
LN+LT	12	37.33	2.61
RN+RT	12	36.92	2.68
LN+RN	12	37.08	3.50
LT+RT	12	38.33	1.87

L, Left; N, nasal; R, right; T, temporal.

It is therefore concluded that on the visual task, if the stimuli are presented to both hemispheres performance is maximized, while if they are presented to only one hemisphere, then the left is capable of processing more of the information than the right.

The differences reported here between the performance of one hemisphere and the other on both tasks can be explained by reference to the specialization of the left hemisphere for the control of speech. In order for digits observed by the right hemisphere to be spoken it is necessary for the right to transmit information to the left. This introduces a measurable delay⁷. In a demanding situation such as this, events occurring during additional transit time could put the right hemisphere at a disadvantage.

It has been shown therefore that when perceptual load is distributed between the cerebral hemispheres, total output is

increased. This result provides further confirmation that each hemisphere possesses its own perceptual analysing system and that each acts as an information channel, while the use of two information channels proves itself superior to the use of one. The presence of two analysing systems showing parallel activity in the brain raises a possibility of the distribution of the perceptual load between conjoint systems, which may be a characteristic mode of brain function not only in split-brain patients but also in normal individuals.

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Chromosome Abnormalities in Severe Protein Calorie Malnutrition

THERE is evidence that DNA replication and protein synthesis are affected when animals are subjected to nutritional deficiency comparable with severe malnutrition in humans¹⁻⁷. There is some evidence of similar abnormalities in children with advanced protein-calorie malnutrition^{1-3,8-11}, which suggests that in these circumstances cell division would fail and chromosomes would become structurally abnormal, either spontaneously or as an exaggerated response to environmental factors such as radiation^{12,13}, chemical agents¹⁴ or viral infection¹⁵⁻¹⁷. We have investigated this possibility with a longitudinal study of chromosome structure in five infants and five preschool children aged 1-60 months with advanced protein-calorie malnutrition (Table 1).

Cytogenetic studies were carried out as usual¹⁸ on the day of admission to hospital and after 15 to 20, 30 to 40, 50 to 60,

Table 1 Characteristics of Patients

Case	Sex	Age (month)	Clinical type of malnutrition	Weight (g)	Height (cm)	Deficit		Diagnostic radiation	Antibiotics *		Sampling time †
						Weight for height (%)	Weight for age (%)		No	Yes	
I	M	60	Kwashiorkor	8.535	74	8%	54%	×	×		I-15-31-40
II	M	15	Kwashiorkor	5.990	66.5	19%	43%	×	×		I-17-37
III	F	15	Kwashiorkor	5.430	71	37%	48%	×	×		I-18
IV	F	14	Kwashiorkor	4.710	65.3	33%	54%	×	×		I-20-40
V	F	12	Kwashiorkor	6.050	69	23%	37%	×	×		I-17-34
VI	M	12	Marasmus	5.310	—	—	45%	×	×		I-15-50-61
VII	F	8	Marasmus	3.865	61.5	36%	54%	×	×		I-17-35
VIII	F	8	Marasmus	5.200	68	34%	37%	×	×		I-18
IX	F	3.5	Marasmus	3.160	61.5	48%	48%	×	×		I
X	M	1	Marasmus	2.430	—	—	40%	×	×		I-17-38-54

* Penicillin-oxacyllin: four patients; nalidixic acid: three patients; colimycin: three patients; kanamycin: two patients; polymyxin: one patient.

† Days after admission. I, Initial sample.

and in one case 140, days. To facilitate the comparison of experimental and control cells blood samples from subjects were mixed with similar samples from normal volunteers of the opposite sex. In this way many environmental variables, such as those due to the medium or related to temperature changes, the presence of mutagens in the blood or in the culture, contamination or culture time²³, are eliminated.

After 72 h 100 cells were analysed from each culture, and abnormalities were classified according to Buckton *et al.*¹⁹. Each assessment was made by the same investigator, who was unaware of the origin of each cell.

Structural abnormalities were classified according to whether one or both chromatids was involved. Abnormalities originating from a double mechanism were counted as one; for example, a dicentric chromosome was considered as such, rather than as a double break. Whenever possible, abnormal chromosomes were classified²⁰. We also made direct chromosome analyses in the bone marrow²¹ of six protein-calorie malnourished children. Abnormalities were classified in the same way as before.

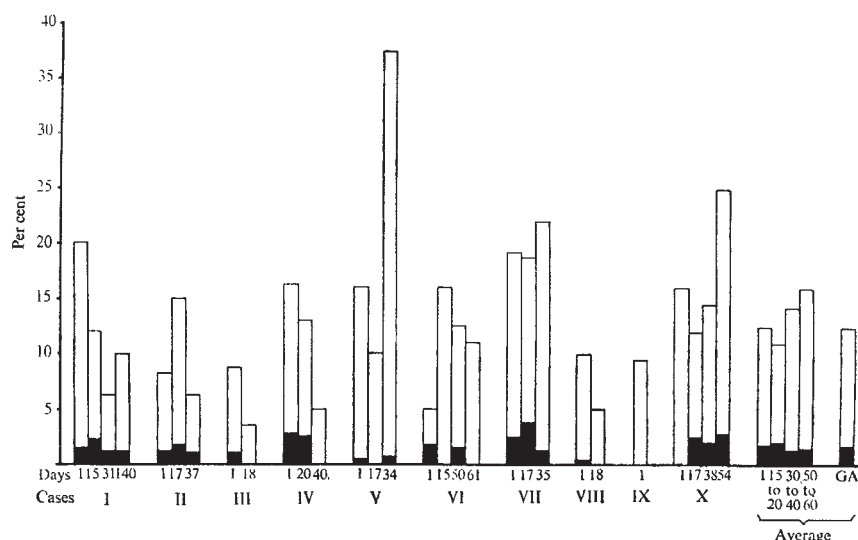
are more frequent than changes of chromosome type; dicentric and ring chromosomes are extremely rare²². Therefore when the effects of mutagens are being investigated, the interpretation of results in terms of the frequency of dicentric chromosomes is preferred to counts of total breaks²².

In our studies, all abnormalities were significantly more frequent in malnourished patients than in controls, but the prevalence of dicentric chromosomes is particularly striking (Table 2). Diccenrics present in five subjects were found in eleven of 2,124 cells. The other abnormalities also persisted with similar frequency throughout nutritional recovery and in one case for 5 months (Fig. 1).

It is impossible to say whether these data are related to malnutrition *per se*, to bacterial and viral infections¹⁵⁻¹⁷ acting synergically with it, to diagnostic radiation^{12,13}, to antibiotics¹⁴ or to all three and possibly other factors acting on malnourished tissues.

Although therapeutic and diagnostic radiation can alter chromosomes¹²⁻²⁸, it is unlikely to have been significant in the production of aberrations in these patients, for only

Fig. 1 Percentage of chromosome abnormalities in each patient until nutritional recovery. □, Malnourished children; ■, controls.



In peripheral blood samples, 2,940 mitoses were analysed, 2,124 from malnourished subjects and 816 from controls. There were significantly more abnormal chromosomes in malnourished individuals (12.1 vs 2.2%; $P < 0.0001$). In most cases only one chromatid was affected; no ring chromosomes were found (Table 2). Five of the ten malnourished subjects had dicentric chromosomes. When initially present, abnormalities persisted in a much higher proportion in malnourished than in control subjects (Fig. 1). As expected, no differences were correlated with sex.

Abnormalities were significantly more frequent in the malnourished children than in the general population²². This was particularly so for dicentric chromosomes, gaps, isogaps and breaks. There were more chromosomal abnormalities in chromosomes of groups G and D, and less in C and F, than expected.

Studies of "spontaneous" aberrations in the general population²² have shown that changes in chromatids, such as gaps,

five of them were subjected to it, and in a very low dose. But any plasma factor able to induce chromosome aberrations^{29,30} would have induced them in the control cells. Moreover, chromosome damage induced by radiation is randomly distributed³¹, which is not the case in malnutrition. The results of our bone marrow study, analysed later, also argue against the influence of radiation.

None of these patients received any one of the antibiotics which seem to be able to change chromosomes¹⁴. We have not the resources to clarify the role of eventual clinically undetected viraemia in the causation of these abnormalities.

Bone marrow was examined for two reasons. First, the persistence of chromosome abnormalities in lymphocytes for up to 60 days may reflect damage incurred in the past, for the life of a lymphocyte may be as long as 1,500 days³². The bone marrow proliferates rapidly and it should reflect events currently in progress. Second, megaloblastic anaemia may be associated with structural chromosome changes^{33,34}. No megaloblastic

Table 2 Structural Abnormalities

	Gaps	Chromatid abnormalities		Total	Dicentric	Chromosome abnormalities			Total	Total abnormalities	Cells analysed
		Isogaps	Breaks			Acentrics	Fragments	Others			
Patients	115	17	46	178	11	4	38	27	80	258	2,124
Controls	5	—	1	6	—	—	5	7	12	18	816

blastic changes were observed in the smears, but direct chromosome analysis of 300 cells revealed 33% of abnormalities (12.3% gaps; 10% isogaps; 2.7% breaks; 0.33% dicentric; 6% acentrics and fragments and 1.7% others).

Suggested causes of chromosome breakage include incorporation of nucleotide analogues into DNA³⁵, linkage of DNA molecules to mutagenic agents³⁶, free radical formation by ionizing radiation³⁹, failure of enzyme processes or of protein synthesis necessary for adequate mechanisms of cellular repair³⁸ or liberation of lysosomal enzymes³⁹. As for protein-calorie malnutrition, either mutagenic factors can more easily produce chromosome changes within a biochemically distorted internal environment, or the repair mechanisms of "spontaneous" breaks are inadequate, permitting the persistence of an abnormally large number of aberrations.

Radiation, viruses and chemical compounds are carcinogenic⁴⁰, and abnormalities of the kind we have described are often found in patients with aplastic anaemia of the Fanconi type⁴¹, with Bloom's syndrome⁴² and with ataxia telangiectasia⁴³, which are all prone to malignancy. Whether malnourished children are abnormally susceptible to haematological malignancy, or whether the high incidence of hepatomas in malnourished Bantu children⁴⁴ or the immunological^{45,46} and haematological characteristics of severe protein-calorie malnutrition are related to our findings remains to be investigated.

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Note added in proof. When seven children from the original group were evaluated cytogenetically 1 yr later, five had attained a normal weight for height. Two culture procedures were used, one using the method already described (A) and another non-mixed (B). Abnormal findings were significantly higher in malnourished than in controls ($P < 0.0001$) and the general population²² (Table 3). No difference was found among the two types of culture suggesting that the technical procedure had no influence on the production of chromosomal abnormalities. No dicentric chromosomes were found, but there was a triradiated figure. In one patient a Ph₁-like chromosome was present in two lymphocytes, but not in bone marrow where 8% of the cells had chromosome abnormalities.

Table 3 Analysed Cells and Chromosome Abnormalities after 1 yr

		Analysed cells	Chromosome abnormalities
Cultures A	Control	106	5 (4.7%)
	Malnourished	244	52 (21.3%)
Cultures B	Malnourished	350	78 (22.3%)
	Total of malnourished	594	130 (21.9%)

Cultures A, malnourished + control.

Cultures B, malnourished only.

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Yawning and Penile Erection induced in Rats by Cortical Spreading Depression

BEHAVIOUR can be stimulated as well as disrupted by cortical spreading depression (CSD)—the massive wave of neural depolarization and subsequent EEG depression which can be induced to spread slowly over a cortical hemisphere by injection of KCl. Eating, drinking and locomotor behaviour have been elicited in rats by administering single waves of CSD (ref. 1). Consummatory behaviour was most likely to be elicited if an animal had extensive feeding experience in the testing chamber. I wish to describe two further effects of single waves of CSD, the induction of penile erections and of stretching and yawning responses.

Waves of spreading depression were generated either by injecting K^+ electrophoretically into the occipital cortices of male hooded rats, or by pressure injection of about 1 μ l. of 6% KCl solution. For electrophoresis, refillable glass cannulae containing 25% KCl and a coiled silver wire were implanted chronically. An anodal current of 1 mA, passed between the wire and an indifferent electrode over the cerebellum for about 10 s, released sufficient K^+ to trigger one wave of CSD (ref. 2). For pressure injection, steel cannulae were placed over the occipital cortices, and KCl was injected from smaller needles attached to flexible polyethylene tubing. The corresponding steady potential changes (SPCs) were recorded bilaterally across Ag-AgCl electrodes placed 2 mm lateral to bregma. The reference electrode was 10 mm anterior to bregma at the midline. The animals were able to eat and drink freely inside a box with one transparent wall. Waves of CSD were administered at least 5 min after any manifestation of the behaviour we were monitoring. Waves were separated by at least 15 min.



Fig. 1 Examples of yawning elicited by waves of cortical spreading depression.

Although much less consummatory behaviour was elicited by CSD than when rats were pretrained to drink¹, the rats yawned frequently after a wave of CSD. Yawning was never observed in the experimental chamber except after CSD. Typically, the response consists of stretching, with extension and raising of a forepaw, accompanied by wide opening of the mouth (Fig. 1). Frequently chewing movements preceded a yawning response by 1–30 s. Yawning was observed in seventeen out of twenty rats tested.

Fig. 2 (top) shows the distribution of onset times of seventy-three cases of elicited yawning in six rats. Of these 25% were elicited

by CSD applied to both cortical hemispheres. This histogram shows the proportion of times yawning commenced in any of the 1 min intervals following injection of K^+ . Onsets were measured from an arbitrary synchronization point of maximal depolarization at the anterior (frontal cortex) recording electrode. Thus, whenever yawning accompanied a wave of CSD, it was most likely to occur first about 5 min after initiation of CSD. The mean number of yawns elicited by each of the seventy-three CSDs was 4.3. Fig. 2 (bottom) shows the distribution of all the 314 elicited yawns. At least three waves of CSD were usually necessary before a rat yawned. On four occasions yawning followed the first wave of CSD, but in each case the animals had experience of CSD on the previous day. Apparently facilitation of yawning after CSD persists for some time. Drowsiness generally coincided with yawning.

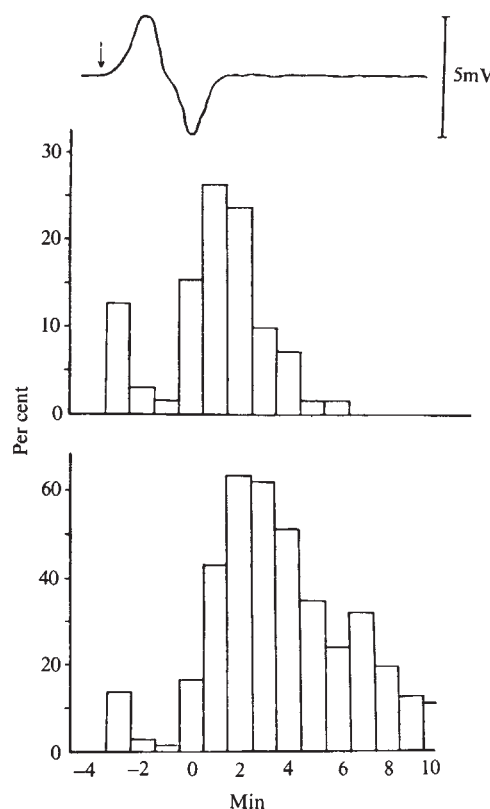


Fig. 2 Top: distribution of onset times of yawning elicited on seventy-three occasions. Bottom: distribution of all 314 yawns elicited on seventy-three trials. Measurements were taken from the peak of the wave of steady potential change corresponding to maximal depolarization at the frontal pole. Arrow indicates injection of K^+ .

When yawning followed waves of CSD it was often possible to elicit the chewing–stretching–yawning sequence by startling the animal with a sudden noise or by tapping on the chamber.

Thus CSD has two effects, namely, repeated waves induce a disposition to yawn, and subsequently single waves can elicit the yawning response. The metabolic, hormonal, and physiological changes which accompany CSD (ref. 3) may provide insight into the central mechanism of yawning, of which little is known⁴.

The distribution of the onset of yawning corresponds closely to the distribution of the other behaviours elicited by CSD in slightly different conditions¹. This fact, in conjunction with the data on the induction of yawning in startled animals, suggests that, given a readiness to yawn, the yawning response occurs as an after effect of arousing stimulation, possibly when the arousal value of the stimulation has dissipated (the latencies of

yawning elicited by startling varied from 1 to 20 s). In such a scheme, a yawn could have some regulatory function in response to a sudden mobilization of neural resources.

During and between bouts of CSD which induced yawning six rats frequently exhibited penile erections. Typically they arched their backs, trembled, reared up on their hind legs, and exhibited a remarkable erection, often with waving of the penis. This was followed by licking and grooming of the genital area. The relationship in these experiments between yawning and sexual excitement is not clear, but it is interesting in the light of recent reports of yawning and sexual arousal induced concurrently by injection of adrenocorticotropin into the brain of rats, rabbits and cats⁵⁻⁷, and by the release of erections and yawning in monkeys in response to their mirror images⁸.

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Monosodium Glutamate induces Convulsive Disorders in Rats

THE physiological and pharmacological effects of L-glutamate have received much attention since its implication in the aetiology of the "Chinese restaurant syndrome"¹⁻³. Although an excess of L-glutamic acid (GA) as the monosodium salt (MSG) was known to cause retinopathy in experimental animals⁴⁻⁷, brain lesions have been noticed only recently⁸⁻¹⁰. There seems to be no agreement about its pharmacological and neurophysiological effects in humans¹¹ and experimental animals¹².

While investigating glucose metabolism¹³⁻¹⁵, we used L-glutamate (as MSG) as a gluconeogenic precursor *in vivo* (unpublished), and observed that rats became somnolent after

intraperitoneal administration of carrier doses. In a study of the effect of L-glutamate on the *in vivo* incorporation of amino-acids into cerebral proteins (unpublished), several animals experienced severe tonic-clonic convulsions, usually between 30 and 120 min after intraperitoneal injection of a large dose of MSG (2 mmol/100 g body weight). Some convulsions terminated in death. We have now made a detailed study of the incidence of seizures following treatment with MSG. As well as taking an electroencephalogram (EEG) during the convulsions, we studied the influence of pyridoxine on the incidence of these convulsive disorders, for pyridoxine is directly involved in the metabolism of GA and gamma-aminobutyric acid (GABA).

We used weanling Sprague-Dawley male rats maintained on either laboratory chow (Teklad), or pyridoxine-control or deficient diets of the following composition: vitamin-free casein, 22%; sucrose, 69%; corn oil, 4%; salt mixture (Northern Regional Research Laboratories), No. 446, 4%; vitamin-fortification mixture (pyridoxine-free), 1%. This basal diet supplemented with 30 mg of pyridoxine HCl/kg served as the pyridoxine-control diet. Controls weighed 380-550 g (10-20 weeks old) and the deficient animals 80-120 g (9-12 weeks old). MSG (2.5 mmol/ml.) was injected intraperitoneally (2.0 mmol/100 g body weight). Pyridoxine HCl (5.0 mg per 100 g body weight) was administered intraperitoneally to some animals 30 min before MSG treatment. For EEG recording, animals were prepared under pentobarbital and chlorprothixene anaesthesia with chronically implanted stainless steel electrodes and then allowed to recover for 1 week before recordings were taken.

Table 1 summarizes the incidence of the various symptoms observed during 2 h after injection of MSG. Marked somnolence was observed in 99% of the animals within 5-20 min. After this (1 h after injection), 52% of the animals salivated copiously, and 31% then displayed what we have described as spastic tremors, varying in intensity from mild to severe myoclonic jerking sometimes followed by vigorous running about the cage and stereotyped biting. This behaviour occurred significantly more often ($\chi^2 = 5.25$; d.f. = 1; $P < 0.05$) in rats on the pyridoxine-control diet pretreated with pyridoxine than in animals on the same diet pretreated with 0.9% NaCl. Seizures, which occurred 30-120 min after MSG injection, were invariably preceded by spastic tremors, and terminated in death in some cases, although the animals usually recovered within 2-3 min and resumed the spastic tremors until the next seizure. The largest proportion of seizures was observed in the animals fed pyridoxine-control diet and pretreated with pyridoxine; the lowest proportion was found in the pyridoxine-deficient and laboratory chow animals, both pretreated with pyridoxine.

To ascertain whether the effects of MSG could be attributed

Table 1 Incidence of Somnolence, Hypersalivation, Spastic Tremors, Seizures and Death in MSG*-treated Rats

Diet	Pretreatment	Somnolence		Hypersalivation		Spastic tremors		Seizures		Death †	
		N	%	N	%	N	%	N	%	N	%
Laboratory chow (21)	Pyridoxine HCl‡	20	95	6	29	5	24	1	5	2	10
Laboratory chow (22)	0.9% NaCl	22	100	16	73	7	32	5	23	6	27
Pyridoxine-control (25)	Pyridoxine HCl	25	100	15	60	15	60	7	28	9	36
Pyridoxine-control (25)	0.9% NaCl	25	100	17	68	6	24	5	20	8	32
Pyridoxine-deficient (15)	Pyridoxine HCl	15	100	4	27	2	13	1	7	1	7
Pyridoxine-deficient (11)	0.9% NaCl	11	100	4	36	2	18	1	9	3	27
Total 119		118	99	62	52	37	31	20	17	29	24

Figures in parentheses indicate the number of animals.

* 2 mmol/100 g body weight, intraperitoneal.

† During 24 h after injection.

‡ 5 mg/100 g body weight.

to the administration of excess sodium *per se* and/or to the tonicity of the solution injected, two groups (eight and four rats respectively) were injected with either NaCl or sodium DL-lactate (2.5 mmol/ml., 2 mmol/100 g body weight). None of the animals developed any symptoms characteristic of MSG treatment.

Of the eleven MSG-treated animals from which EEG recordings were taken, five displayed tonic-clonic convulsions during the 2 h recording session. The seizures were characterized by high amplitude spikes typical of this form of convulsion.

Thus MSG in a dose smaller than that used by Olney⁸ in adult mice, and by Adamo and Ratner¹² in infant rats, can induce tonic-clonic seizures in adult rats. Administration of pyridoxine to animals which already had an excess of this vitamin (30 mg/kg diet) significantly increased the occurrence of spastic tremors. It should be noted that we used intraperitoneal administration rather than the subcutaneous method of the previous authors.

The types of seizure seen in the rat are remarkably similar to those noted by Wiechert *et al.*¹⁶⁻¹⁸ in dogs and rats after intracisternal injection of L-glutamate (but not other amino acids), and pyridoxal-5'-phosphate (PLP). Wiechert *et al.*¹⁶⁻¹⁸ and Elliot and van Gelder¹⁹ have suggested that the balance between GA and GABA but not the absolute amount of GABA *per se*²⁰ is a decisive factor in the control of cerebral neuronal activity. Unphysiological shifts in this balance have been implicated in the onset of seizures. Our findings suggest that intraperitoneally administered L-glutamate in the dose used may cross the blood-brain barrier, which would affect the steady state levels of GA and GABA, thus inducing seizures. The effect of pyridoxine, on the other hand, may be a consequence of differential stimulation of the two enzyme systems—GA decarboxylase and GABA aminotransferase—the latter having a higher affinity for PLP²¹. The resulting change in the relative concentrations of GA and GABA may determine the seizure activity. Other possibilities such as a disturbance in the electrolyte balance do exist and a detailed neurochemical study of L-glutamate-induced convulsions is necessary.

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Handedness not a Function of Birth Order

It has been suggested¹ on the basis of a survey of 648 university students (95 left handed = 14.7%) that handedness is a function of birth order and that left handedness may be caused by neurological insults associated with prenatal or birth trauma. I here report a similar survey of 974 university students (141 left handed = 14.5%).

Handedness was classified as in the original investigation¹ by asking each subject "Which hand do you use for writing?". As expected, the population of left handed students included a high proportion of twins ($P < 0.01$) and a nonsignificantly smaller proportion of women than did the population of right handed students.

Table 1 Handedness and Birth Order and Parental Handedness

Birth order	R	L	Parental handedness	R	L
1, 4+	445	62	R, R	722	117
2, 3	388	79	RL, LR or LL	98	23
	$\chi^2 = 4.31$			$\chi^2 = 2.18$	
	$P = 0.05$			$P = 0.15$	

Table 1 shows the relationship between handedness and the two categories of birth order—high risk (first and fourth or later birth) and low risk (second or third births). For the whole group there are significantly more ($P = 0.05$) left handed persons in the low risk category, the exact opposite of the previous findings¹. If the results of these and previous investigations are combined, there are almost equal numbers in both risk categories for both right and left handed series. These results strongly suggest that the apparent effect of birth order¹ on handedness is simply a sampling error.

It is more usual to attribute handedness to inheritance², although opinion is divided about the nature and strength of the effect³. In this study, 960 of the students were able to answer the questions "What hand does your mother write with?" and "What hand does your father write with?". Sometimes it was reported that one or the other parent wrote with the right hand now but changed in grade school. Such parents were counted as "left". Table 1 shows the distribution of parental handedness of the right and left handed students. None of the 140 left handed students had two left handed parents although three right handed students did. Although the proportion of left handed students with one left handed parent (16.5%) was slightly larger than the proportion of right handed with one or two left handed parents (12%), the

difference was not significant. The result is thus similar to previous studies³ which indicate that a familial effect can be detected only in very large surveys.

Left handedness is known to be but one of a spectrum of effects related to cerebral dominance, and it is thus perhaps relevant that when asked "Have you ever taken part in any form of speech therapy?" and "Do you find it easy to make diagrams and sketches?", left handed students were found to answer "Yes" significantly more often ($P < 0.01$) than did right handed. When the male and female populations were separately examined it was found that the significant effects were associated with the female left handed population ($N = 51$) in the case of speech therapy ($P = 0.015$) and the male left handed population ($N = 90$) in the case of drawing diagrams ($P = 0.005$).

The data thus support an explanation of handedness as one expression of cerebral dominance but throw no light on the problem of the determination of the side of dominance.

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Excess of Like Sexed Pairs of Dizygotic Twins

BEFORE recent developments with marker genes, it was not possible to assign with certainty the zygosity of a substantial proportion of twin pairs. So it was usual, when estimating the incidence of monozygotic (MZ) and dizygotic (DZ) pairs, to use Weinberg's differential rule¹, which postulates that among DZ pairs the frequencies of same sex and of opposite sex pairs are almost exactly equal (the difference from equality being occasioned by an adjustment for the sex ratio). Although it is important to know whether this is true, as far as I know, it has never been adequately tested, though the suggestion has been made more than once that estimates (based on Weinberg's rule) of the frequency of MZ twins seem to be too high^{10,15}. I have some suggestions to explain this disparity.

I wish to suggest that either the rule is seriously in error (like sex and opposite sex DZ pairs being distributed roughly in the ratio of 7 : 6) or that blood typing is less efficient than is generally thought.

In principle, the point can be tested quite easily with a large sample of twins, for each member of which data are available about sex and about one or more markers. Two criteria must be observed. First, the sample of twins must be random with regard to sex composition. This condition is most easily fulfilled if they are ascertained at birth. Second, all the twins in each sample must be tested on the same set of markers. Then for a given set of material, the data can be thrown into the four-fold Table 1.

I have located eight sets of data which bear on the accuracy of Weinberg's rule. First there are the data of Simmons *et al.*², which were estimated to contain forty-five same sex and twenty-five opposite sex DZ pairs. This, however, may be a non-random sample. Index twins were ascertained by their presence (due to illness) in a hospital, and there could have been (as Simmons *et al.* suggest) greater difficulty in locating

(for purposes of blood typing) a co-twin of the opposite sex than of the same sex. Next there is the Birmingham twin survey in which Cameron³ suggested that there was an excess of same sex pairs among the DZ twins, but did not give the exact figures.

The third set of data comes from Nylander⁴, and concerns 608 pairs of twins born in Aberdeen. Among these, 198 pairs were recorded at birth as being of opposite sex and 404 pairs were of the same sex, while the sex of six pairs was not recorded. Of the 404 pairs of same sex twins, 233 (57.7%) were later ascertained for blood typing at ages from 9 months to 15 yr. Of these 233 cases, 131 were proved to be DZ. In addition, there were 102 pairs who were concordant for both sex and blood. Using the tables of Smith and Penrose⁵, one would estimate that about five of these were DZ too. Thus altogether there were about $131 + 5 = 136$ same sex DZ pairs later ascertained for blood typing.

Table 1 Data for a Large Sample of Twins

Markers	Sex			
	Concordant		Discordant	
	Concordant	<i>a</i>	<i>b</i>	<i>d</i>
	Discordant	<i>c</i>		

If there had been wastage among the opposite sex twins at the same rate as among the same sex twins, only 57.7% of them (114) would have been available for comparison. So the suggestion is that among DZ twins, the ratio of the frequencies of same sex pairs to opposite sex pairs in these data was about 136 : 114.

In the fourth set of data¹⁵ there were also estimated to be more same sex than opposite sex DZ twin pairs. The rest of my eight sets of data are found in refs. 6-9. Dr Corney informs me that the material presented in ref. 9 contains all that discussed by Nylander and Corney¹⁰ with some additional data. These four sets of data all seem to satisfy my two criteria of randomness of ascertainment and identity of markers.

Table 2 DZ Twin Pairs

Reference	Same sex			Opposite sex		
	<i>c</i>	$e = cb/d$	$f = c + e$	<i>b</i>	<i>d</i>	$g = b + d$
6	79	20	99	18	71	89
7	38	12	50	7	22	29
9	252	39	291	32	208	240
8	84	1	85	1	88	89
	453		525		389	447

Table 2 shows the frequencies observed in the cells *b*, *c* and *d* (Table 1) in each of these studies, and frequencies derived from them. It is not appropriate to test the difference between the two totals *f* and *g* by χ^2 because a significant result might simply be the consequence of the inefficiency of the method of estimating the frequencies *e*. Indeed, these latter frequencies are estimated from the observed frequencies *b* with which they are compared above, so the comparison (between *e* and *b*) can offer no information. So the only comparison which can usefully be made is between the frequencies of pairs who are concordant and discordant for sex among those who are discordant for markers, that is between *c* and *d*. These two frequencies are respectively 453 and 389.

The significance of the difference between these two observed frequencies is measured by $\chi^2 = 4.86$, $P < 0.03$, and this tendency is confirmed strongly by all the other (perhaps less reliable) data^{2-4,15}.

This apparent excess of same sex DZ twin pairs cannot result simply from the fact that the sex ratio is not unity but

about 106 males to 100 females at birth. For then, if the sexes of members of DZ pairs were independent of one another, the proportion of same sex pairs would only exceed 0.5 by 0.00045.

The frequent suggestion that the male foetus is at higher risk of mortality than the female^{11,12} is not likely to explain these findings; it has been disputed¹³, and moreover there is doubt whether the sexes are at different levels of risk when only twins are considered¹⁴. In any case the mortality differential would have to be far greater than seems credible to account for the present results.

Renkonen¹⁵ thought that the discrepancies between observed data and those expected on Weinberg's rule might be explained by the fact that there is a higher mortality of MZ than DZ twin foetuses. Cannings¹⁶, however, has proved mathematically that this argument is invalid. It is possible that among DZ twin foetuses, opposite sex pairs are at greater risk of mortality than same sex pairs. I can think of no ground for this belief: and Dahlberg¹⁷ considers it to be false.

I have reviewed elsewhere the evidence for the thesis that the sex of offspring is related to the cycle day of conception¹⁸. It seems to me that this evidence suggests that, on the average, male zygotes are formed earlier in the cycle than female. If this were true, then the foregoing results on the sex distributions of DZ twin pairs would be at least partially explained, the same sex pairs being formed disproportionately often at either end of the fertile period and opposite sex pairs (less frequently) in the middle. If blood typing were not 100% efficient, then occasional MZ pairs would be misclassified as same sex DZ pairs, but no comparable error is likely to lead to a same sex DZ pair being misclassified as MZ.

When twins are blood typed at birth, those classified as DZ seem to show an excess of same sex pairs. This may be due to errors in blood typing, or the possibility that male zygotes are formed earlier in the menstrual cycle than females. The second of these possibilities throws some doubt on Weinberg's rule.

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Supernatural Beliefs among Graduate Students at the University of Pennsylvania

JAHODA¹ at the University of Ghana and Pasachoff *et al.*² at Harvard University have tested the hypotheses that (a) general university education leads to a decline in supernatural belief, and (b) scientific training in particular hastens this decline. Neither of these hypotheses was supported by the data presented. In both studies, however, only male undergraduates were examined. This suggests a natural extension of the basic paradigm to graduate students and female students. Examination of graduate students' beliefs is a further test of both hypotheses because graduate students have had both more university education and more experience and training in their particular field, for example, science. The addition of female students eliminates the possible bias effect due to testing of one sex only. This extension was carried out at the University of Pennsylvania.

One hundred subjects living in an apartment building exclusively for graduate students and their spouses were interviewed about the degree of their belief in astrology, extra-sensory perception (ESP), flying saucers (UFOs), the power of prayer, witchcraft and the existence of an evil force, a supreme being and a personal god. The first four of these were taken from Pasachoff *et al.*² and the question on witchcraft from Jahoda¹. All questions were of the form, "Do you believe in the existence of an evil supernatural force?" or, "Do you believe that witchcraft has any supernatural power?" Personal interviews were used rather than the simple questionnaires of the earlier studies, because Pasachoff *et al.*² reported problems due to ambiguity of phrasing. Our interviewers were instructed to explain the questions if problems arose. Two subjects were eliminated from the analysis because they were not graduate students. The belief scores of the remaining ninety-eight were adjusted to a scale of 20, where 0 denotes "total unqualified disbelief" and 20 denotes "total unqualified belief"¹. This made the data comparable to those of the original studies. Cell frequencies do not in every case add to the total because some data were improperly transcribed.

Table 1 Average Belief by Year of Graduate Study

Question	First year	Second year	Third or more years	All years
Astrology	4.37	4.00	3.00	4.16
ESP	12.38	12.91	10.00	12.24
UFOs	7.31	6.55	5.00	7.14
Prayer	7.79	5.64	7.40	7.68
Witchcraft	4.19	5.09	2.40	4.12
Evil force	3.61	2.73	2.80	3.40
Supreme being	11.92	12.36	12.00	12.04
Personal god	9.95	5.45	8.80	9.56
Mean	7.69	6.84	6.43	7.54
No. of subjects	75	11	10	98

Scores are means of belief over the indicated number of subjects, where 0 equals total unqualified disbelief, and 20 equals total unqualified belief.

The results are strikingly similar to those of the other studies, a finding which in itself discredits hypothesis (a). The overall mean belief score of 7.54 in this study is within one point of the comparable means in the earlier studies. Although the means for each year of study do indicate a trend in the predicted direction of decreasing belief over time, the differences are small and, by the Kendall coefficient of concordance (W), are non-significant. The value of W is 0.25, whereas values of

$W > 0.38$ are required for significance at the 0.05 level. We found no firm support therefore for the hypothesis that general education leads to a decline in supernatural beliefs.

The results ordered according to field of study differ somewhat from those of the earlier studies. Jahoda¹ reported belief scores for scientists falling between a high for students in the arts, and a low for students in social studies. Pasachoff *et al.*² reported no clear differentiation by field, although scientists generally had lower mean scores than students of the other fields. In this study, however, natural and biological scientists had the highest mean belief scores and students in the arts (humanities) had the lowest.

Table 2 Average Belief by Field of Study

Question	Humanities	Soc. sci.	Nat. sci.	Biol. sci.	All fields
Astrology	4.14	3.84	5.25	3.90	4.16
ESP	13.00	11.44	13.75	12.16	12.24
UFOs	5.88	7.00	5.25	8.30	7.14
Prayer	5.20	8.50	5.00	8.20	7.68
Witchcraft	2.94	4.34	5.25	3.54	4.12
Evil force	3.06	2.22	4.75	4.44	3.40
Supreme being	10.40	12.70	12.00	12.16	12.04
Personal god	7.20	9.54	13.00	8.14	9.56
Mean	6.48	7.45	8.03	7.61	7.54
No. of subjects	15	37	8	37	98

Application of the Kendall test, however, proved these differences to be insignificant ($W=0.13$), values of $W > 0.32$ being required for significance. Thus the hypothesis that scientific training leads to a greater reduction in supernatural belief is neither supported nor refuted.

Differences in the data arranged according to sex are irregular and the column means are nearly identical. This suggests that belief in the supernatural is not affected by innate or environmental differences attributable to sex differentiation, at least among graduate students. The sample of female students, however, was small.

Table 3 Average Belief by Sex

Question	Male	Female	Total
Astrology	3.68	6.00	4.16
ESP	12.00	12.66	12.24
UFOs	6.86	7.04	7.14
Prayer	7.64	7.64	7.68
Witchcraft	4.26	3.04	4.12
Evil force	3.56	2.54	3.40
Supreme being	11.60	13.44	12.04
Personal god	9.40	9.82	9.56
Mean	7.38	7.65	7.54
No. of subjects	73	22	98

This extension of earlier questionnaire research to graduate students and female students indicates that university education in all fields has little effect on the supernatural beliefs held by male and female graduate students. We suggest that the next step would be a long term study of the belief changes in a heterogeneous college population, rather than comparing the beliefs of students at differing levels and fields. In any event, the

striking similarity of the overall results in three different populations indicates a real phenomenon for further study.

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Vascularization of Skin Grafts

THE question of how skin grafts are revascularized is still not resolved¹⁻³. In particular, it is not known whether the vessels in the vascular bed of a graft derive from the host or the donor. In what follows, we provide three independent lines of evidence which suggest that in full-thickness skin grafts, the vessels are native to the graft at least for the first several days and during the period of allograft rejection. The possibility that the endothelium in surviving allografts may eventually be replaced by cells of host genotype is important⁴ but outside the scope of this report.

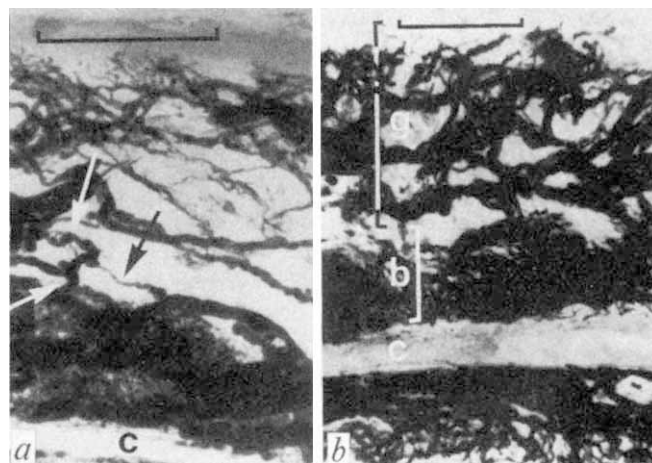


Fig. 1 Microangiograms demonstrating the extent of vascularization of (a) a 3 day and (b) a 2 day old allograft. The appearance of the graft vasculature does not indicate its derivation from newly grown-in host capillaries. It is much more likely that the discrete vessels, seen to bridge the space between graft and graft bed (arrows), connect the graft's native vessel network with that of the host in the graft bed. g, Graft; b, graft bed; c, cartilage.

Rabbits were used for the study of both autografts and allografts. Skin disks of full thickness and 1.7 cm in diameter were obtained from the dorsum of the ear by sharp dissection, fitted into defects similarly created and sewn into place with at least eight fine silk stitches.

Grafts were microradiographed after filling the ear vascular bed, including capillaries, with a radio-opaque mass, introduced into a host artery⁵. The resulting microangiograms showed without exception, in more than fifty experiments, that both autografts and allografts are in extensive vascular continuity with the host within 48 h. The following characteristics point to graft vessels being preformed, and therefore of graft origin: (a) at 48 h they already seem to be part of a mature

vascular bed; vessels extend throughout the thickness of the graft, and are of various calibre, indicating differentiation; (b) some well defined individual vessels connect host vessels with vessels in the graft across the graft/host interface (Fig. 1). These connecting vessels do not have the microangiographic appearance of invading capillaries. It is highly unlikely that the entire visualized vascular bed of 48 h old grafts could have developed from these relatively few bridging vessels in this short time. Capillary growth in the mouse, observed under the microscope in connexion with graft vascularization, proceeded only at a rate of 5 $\mu\text{m}/\text{h}$ (ref. 6). At this rate, a graft of approximately 1 mm thickness could not be expected to be vascularized to the extent shown in Fig. 1 in less than 8 days.

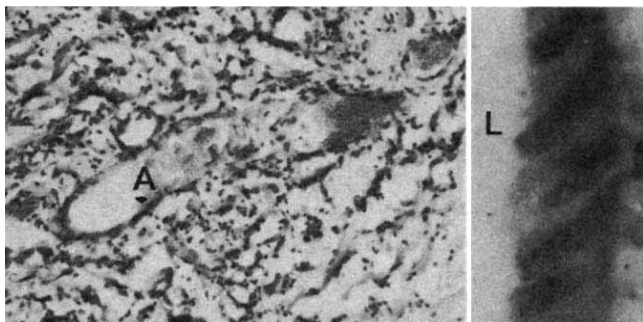


Fig. 2 A fully differentiated artery (A) in a 2 day old skin graft, which is in functional continuity with the host's vascular system as indicated by the presence in its lumen of barium sulphate. To the right is a high power view of the muscular wall. L, Lumen.

The presence in grafts of differentiated vessels in functional continuity with the host vasculature was confirmed histologically. Fully formed arteries, readily identified by their muscular coat, and filled with injection mass, were seen in 2 day old grafts (Fig. 2). In the light of a previous study of the rejoining of divided vascular beds and of the differentiation of arteries from a primitive capillary network⁷, this degree of differentiation within 2 days seems unlikely, especially if vascular differentiation is considered to represent a functional adaptation to a given haemodynamic situation. It has been demonstrated convincingly that major arteries, native to grafted skin, can become incorporated into the ultimate vasculature of a graft⁸.

To give an indication of the origin of the vasculature, the skin was labelled locally with ³H-thymidine, either by intradermal injection or by intra-arterial infusion⁹. In several experiments 4 day old skin allografts were labelled by a single intradermal injection (5 μCi in 0.05 ml. of saline) 2 h before they were returned to the original donor as autografts. This assured widespread labelling of endothelial cells, for in grafted skin they proliferate extensively⁹. Labelled skin was grafted to unlabelled hosts, and unlabelled skin was used to cover the defects created on labelled ears. From 2 to 7 days after grafting, autoradiographs of at least four widely spaced sections of grafts and their beds were prepared by the dipping technique, using Kodak NTB2 emulsion. Exposure was for 8 weeks. Labelled skin was accepted like normal skin, and continuity of graft vessels with those of the host was angiographically demonstrable by 48 h after grafting. Autoradiographs of prelabelled skin revealed that vessels, functionally continuous with the host vasculature, were lined by densely labelled endothelial cells (Fig. 3). This clearly marks them as of graft origin. That they were alive is indicated by the absence around them of diffused-out label, and by their incorporation of ³H-thymidine when labelled directly in such grafts¹⁰. In the graft bed, many endothelial cells as well as fibroblasts and other cells were weakly labelled. These cells, not derived from primary labelled graft cells, have taken up small amounts of label

released from labelled graft cells⁹. Conversely, no well labelled endothelial cells were seen in vascularized grafts of unlabelled skin placed on prelabelled graft beds containing many heavily labelled endothelial cells. Light labelling of graft endothelial cells, in common with that of epithelial cells and fibroblasts, did occur and, as in the reverse case, was attributable to uptake of label released by labelled cells. This absence in grafts of endothelial cells identifiable as descendants of local host endothelium is further evidence against vascularization of grafts by host capillaries. It is unlikely that the complete absence of densely labelled endothelial cells in these grafts was due to dilution of label in consequence of a sustained rapid proliferation, for that would imply that within the limits of the sections examined not a single heavily labelled endothelial cell, once in the graft, could have stopped dividing before a certain time. The identification of graft endothelial cells as of donor origin precludes, at least in the rabbit, a mechanism of vascularization observed in the mouse, in which ingrowth of host vessels is said to occur along pre-existing graft vessels, which are used as mere non-viable conduits⁶, thereby preserving the architectural characteristics of the graft vasculature.

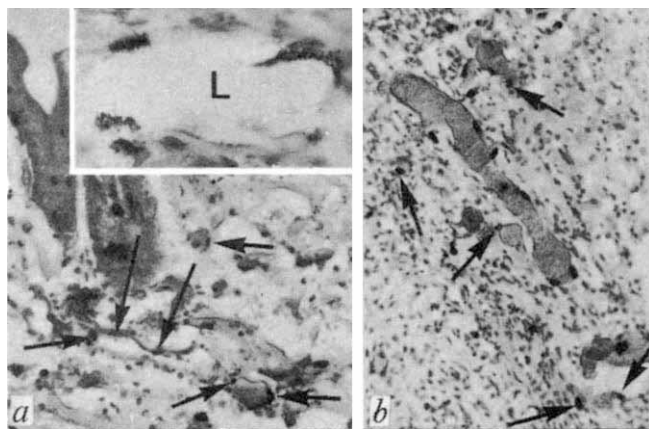


Fig. 3 Autoradiographs showing vessels within two 2 day old autografts, near the surface of one graft (a) and deeper in the dermis of another (b). Inset: high power view of a single vessel showing the individual silver grains overlying labelled endothelial cells, which in the lower power views appear as black blobs and of which some less readily perceived ones are marked by arrows. These grafts were labelled while resident on an allogeneic host before being returned to their sites of origin. The presence in these grafts of densely labelled endothelial cells lining vessels in continuity with the host vasculature strongly suggests that these vessels were preformed and hence of graft origin. L, Lumen.

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BOOK REVIEWS

Pakistan Geology

Stratigraphic Boundary Problems: Permian and Triassic of West Pakistan. Edited by Bernhard Kummel and Curt Teichert. (Department of Geology, University of Kansas, Special Publication No. 4.) Pp. 474. (University of Kansas: Lawrence and London, December 1970.) \$25.00.

THIS book is an expansion of a summary by Kummel and Teichert (*Neues Jharb. Geol. Pal., Abh.*, 125, 297; 1966). It starts with a paper of 110 pages by Kummel and Teichert dealing with general geology, history of research, stratigraphy as defined by the study, comparative palaeontology, conclusions, the full descriptions of twelve measured sections and a list of all sample localities. This is followed by seven papers, each by a specialist, systematically describing the ammonoids, brachiopods, ostracods, conodonts, achritarchs, and tasmanitids with one paper on palynology. Conclusions are drawn; the papers are well illustrated and all conform to a high standard.

The volume forms part of a re-study of the problems connected with evolutionary changes that took place among marine organisms at the passage from the Palaeozoic to the Mesozoic. It is pointed out that most authors who have considered this contact have emphasized the factor of suddenness in the change-over of marine forms.

One sees the same suddenness operating in the changeover from Mesozoic to the Tertiary and, in view of the abundant literature concerned with this, may wonder why published works on the Permian-Triassic boundary in marine facies seem so rare. As the authors point out, the answer is to be found in the scarcity of sections where marine conditions persisted across the boundary. Their worldwide survey revealed only five areas where such marine sections are known to occur: Southern China, Kashmir (to which Spiti can be added), Salt Range, Azerbaidjan-Armenia and North-East Greenland. In choosing the Salt Range for the initial study, Kummel and Teichert have selected a standard, or type, area fundamental to the understanding of the geology of the Indian subcontinent and it is important to note exactly where they have placed the Permian-Triassic boundary and for what reason.

Formerly it was generally accepted that the boundary fell between the Upper Productus Limestone and the Ceratite Limestone. The authors have split the Upper Productus Limestone into two units: a lower Chhidru formation (alternating sandstone and limestone) and an upper Kathwai member (dolomite and limestone). The Kathwai becomes the basal member of Gee's Mianwali formation of which the middle, or Mithwali, member is the former Ceratite Limestone.

The first appearance of the ammonoid genera *Glyptopliceras* and *Ophiceras* at the base of the Kathwai member is taken to mark the base of the Triassic. A similar boundary can be found in Kashmir and in North-East Greenland.

The authors have thus placed the boundary lower than that commonly accepted previously. They also mention that the new boundary coincides with a lithological change from a white sandstone, forming the top of the Chhidru, to a dolomite which is basal Kathwai.

I believe that this whole work forms an important contribution to the understanding of evolutionary changes at the Palaeozoic-Mesozoic contact and it is to be hoped that a study of equal quality may soon be made in Kashmir and Spiti where comparable marine sequences exist.

J. M. DORREEN

Victorian Attitudes

Victorian Science: a Self-Portrait from the Presidential Addresses of the British Association for the Advancement of Science. Edited by George Basalla, William Coleman and Robert H. Kargon. Pp. 510. (Anchor: Garden City, New York; Bell: London, February 1971.) £1.20.

Victorian Attitudes to Race. By Christine Bolt. (Studies in Social History.) Pp. xviii+254. (Routledge and Kegan Paul: London; University of Toronto: Toronto, January 1971.) £3.

BEATRICE WEBB once asserted, without necessary qualifications, that one of the most widely held assumptions of the mid-Victorian period was that "physical science could solve all problems". However naive and threadbare this doctrine might sound today, we would be wrong to think that two world wars and several decades of social evolution

have led to a wiser estimate of the role of science in society. In fact, the remarkable thing about the two works under review is the extent to which they show continuities between the Victorians and ourselves.

We should be most grateful to Messrs Basalla, Coleman and Kargon, whose editing of presidential addresses to the British Association does provide an admirable self-portrait of Victorian science. With the BA now in such desperate financial (and intellectual) straits, this collection almost reads as a eulogy to a once-powerful institution. The primary tasks which the association set for itself in the nineteenth century were those of popularizing scientific knowledge and professionalizing scientific activities. The link between these two activities was money, of which there was little available for serious research. If the boundaries between altruism and self-interest were as ill-defined then as they are now, at least the great Victorian scientists were largely untroubled by second thoughts as to whether or not their calling was the engine of social progress.

The concerns and intellectual achievements of the period's "cultivators of science" are well documented in *Victorian Science*. By providing short commentaries on each of the speeches, the editors have succeeded in making their book suitable both for the general reader and for initiates into the history of science. Their introduction, however, does not plumb very deeply into the sociology of nineteenth century scientific life. In particular, it fails to indicate convincingly—beyond a few allusions to evident scientific and technological advances—why the Victorians seemed to be so enamoured of science in the first place.

One hypothesis might be that, for the life sciences at least, the absence of a sharp dividing line between "scientific" and social thought encouraged a pattern of mutual influence between scientists and social philosophers. To the extent that popular (and political) metaphors found their way into scientific laws, the practising scientist was helping to reinforce "non-scientific" world views. Much of R. M. Young's recent work on the Darwinian debate has served to highlight some of these connexions.

Further evidence for this contention can be found in Christine Bolt's *Victorian Attitudes to Race*. In her open-

ing chapter on "The Scientific View", Dr Bolt provides numerous examples of British anthropologists whose pronouncements on subject races differed little in content from those of racist apologists for imperialism. Indeed, at least one distinguished Victorian scholar in this area (James Hunt) was quite explicit on the need for further anthropological studies as an aid to consolidating the Empire's rule in the colonies. Although the author is well aware of the ideological component of her subjects' work, her separation of the "scientific view" from the rest of her material suggests a too optimistic judgment about the later attempts of life scientists to free themselves from racial prejudice. Reference to the early history of the Eugenics Society would have been a useful corrective here, not to mention the recent and continuing Jensen controversy.

Yet in all fairness it must be said that the attitude of scientists is a subject subsidiary to Dr Bolt's chief interest, namely the description of Victorian public opinion on the subject of race. Until recently there has been a dearth of detailed historical studies on this question. Along with Victor Kiernan's *The Lords of Human Kind*, her book provides us with the kind of background data necessary for an understanding of both Victorian and contemporary race relations. That it can illuminate such important subjects as the British reactions to the American Civil War, the Jamaica Revolt and the Indian Mutiny goes without saying.

After what must have been a long and arduous period of investigation, the author, somewhat surprisingly, ducks the task of providing a conceptual framework for explaining her material. Presumably there will be a number of armchair theorists who will be only too eager to do this for her. But I think this is a pity, since her knowledge of the sources will necessarily be unrivalled in terms of depth and subtlety. Instead she leaves us with what really are platitudes about the relationship between mental rigidity and prejudice and about the need for "common sense" about race. Dr Bolt's evidence is so much weightier than her conclusions that we can only hope for later theoretical reflexions on her part.

P. G. WERSKEY

Raman Spectroscopy

Raman Spectroscopy: Theory and Practice, Vol. 2. Edited by Herman A. Szymanski. Pp. ix+221. (Plenum: New York and London, 1970.) \$17.50.

THIS book follows a first volume published in 1967 which included a series of chapters written individually by experts on the theory of the Raman

effect, the spectra of solids, Raman intensities and the impact of the laser on Raman spectroscopy. Volume 1, although useful, suffered very badly from lengthy delays in production and therefore the advent of the second volume has been awaited with some anticipation. The second volume contains a series of chapters by such distinguished contributors as L. A. Woodward, A. C. Albrecht, H. W. Schrotter and R. E. Hester. Most of the authors are new to the series, only Dr Woodward and Professor Hester having contributed to the previous volume.

The first chapter, on vibrational selection rules and polarization by Dr Woodward, maintains the excellent scientific and literary standard set by him in his introduction to the first volume. As an account of the principles behind structure determination, based on the comparison of infrared and Raman spectra, this chapter will certainly become a classic reference since it not only surveys the method but emphasizes the advantages and limitations inseparable from this type of work. Developments in the theories of vibrational Raman intensities are reviewed clearly and concisely in a chapter by Dr J. Tang and Professor Albrecht. Since there is a dearth of reviews in this area, Tang and Albrecht's contribution is most valuable. This chapter is followed by one on Raman spectroscopy with laser excitation by Dr Schrotter. In a volume published in the 1970s this seems a little out of place, for by 1967 it was clear that the discharge lamp was no longer to be considered seriously as a Raman source. Fortunately, Dr Schrotter gives a considerable amount of experimental technique in his article and this is certainly useful, if a little dated. The second part of his chapter, however, which sets out to describe some of the experiments feasible as the result of the use of the laser as a source, although good reading, is not intended to be a review and would seem to be a sermon aimed at the converted.

Dr L. A. Blatz contributes an article describing experiments aimed at determining low frequency Raman spectra of liquids. This area of study has been completely revolutionized experimentally by the use of laser excitation and in particular by the use of single mode argon ion lasers operating at 5145 Å and iodine vapour filters, yet these techniques are not discussed and the chapter is largely of historic interest. In particular, little or no detail on Rayleigh wing, Brillouin or laser-beat spectroscopic methods appears and this is indeed disappointing as a critical review in this area is urgently required.

Professor Hester's contribution on Raman spectra recorded at high and low temperatures is reasonably topical and

contains a considerable amount of experimental information and a number of examples of the applications of the techniques described, for example, to molten covalent inorganic compounds and salts and also to powders and crystals at low temperatures. The final chapter on poor scatterers by Dr E. Steger is a little mysterious; much of the experimental information is obsolete or is covered elsewhere in the book while the short review section simply contains a random selection of examples of the spectra of compounds with weak bands or of dilute solutions.

I found this book disappointing because it seems to have suffered the fate of its predecessor and taken too long to prepare. Further, the editor does not seem to have edited the manuscripts provided, or to have guided his contributors. One cannot criticize an author in this type of work for being unaware that he is repeating information contained in another chapter but the editor should have spotted these duplications. Further, it is by no means clear what the editor intended to achieve in his book. At a cost of over £5 it is hard to recommend this volume to personal buyers but it would make a useful addition to a good scientific library. It is tacitly assumed that further volumes in the series will appear eventually; let us hope these can be produced quickly, that an attempt will be made to update obsolete articles in earlier volumes and that the editor will wield a heavy pencil.

P. HENDRA

Organic Electrochemistry

Electrochemical Reactions in Non-aqueous Systems. By Charles K. Mann and Karen K. Barnes. (Monographs in Electroanalytical Chemistry and Electrochemistry.) Pp. viii+560. (Dekker: New York, October 1970.) \$34.50; £16.40.

IN the past decade, there has developed a clear need for an authoritative and well balanced text on organic electrochemistry to provide a common base for both the organic chemist and the electrochemist working in this area. Unfortunately, neither this book nor the other recent texts by organically inclined authors come close to filling the gap. Indeed, this volume is concerned essentially with the electrochemistry of organic compounds in non-aqueous media; the single final chapter on inorganic compounds provides insufficient justification for a wider claim. An introductory chapter on the interpretation of electrochemical measurements is included to help readers who have insufficient backgrounds in electrochemistry. It is too short to be of much value in this respect, and its main utility is in

giving data on the behaviour of background electrolytes and reference electrodes in a number of solvent systems.

The authors state that the book is based on a literature search covering the period to the end of 1968. The result is a systematic but rather uncritical review of this literature together with a large and useful compilation of the electrochemical reaction potentials of many compounds. There are chapters dealing separately with particular classes of compounds and no further attempt is made to show the underlying unity of the subject nor to discuss the many urgent and unanswered questions in the field. One looks in vain for the authoritative touch, the panoramic view that might delineate the skeletal structure on which the great mass of data can be hung, so that the basic chemistry is emphasized. Thus the reader is neither stimulated nor guided but merely informed. It must be conceded that the information provided will be of great use to research workers in the field, and the publishers' claim that the book serves as a reference and guide to the literature is well justified. This function is, however, vulnerable in view of the rapid development of the subject since 1968 and in many respects the book is already outdated. Thus one questions the whole concept of producing a review of this type in book form rather than as a regularly updated series in the review literature. A glance at the price strongly reinforces this view.

A. BEWICK

Useful Mathematics

Low Energy Hadron Interactions: Invited Papers Presented at the Ruhestein Meeting, May 1970. (Springer Tracts in Modern Physics: Ergebnisse der Exakten Naturwissenschaften, Volume 55.) Pp. v+290. (Springer-Verlag: Berlin and New York, 1970.) 78 DM; \$21.50.

THIS volume consists of papers, chiefly of a review nature, given last year at a specialized meeting on low energy interactions of strongly interacting particles. They describe investigations in elementary particle physics which are more humdrum—and more typical—than the occasional sensations which reach the popular press.

During the past twenty years countless experiments at all the large accelerators have measured the cross sections for pions, nucleons, kaons, and photons scattering off nucleon targets. Dynamical attempts to calculate these cross sections have had very limited success, with the result that many theorists have become more modest in their ambition, and content themselves with fitting experiment by parametrizations incorporating the best founded theoretical constraints, but with little predictive

power. The aim is to determine masses, coupling constants, and scattering lengths, which are then treated as data to guide the construction of a proper theory.

Many such analyses have been performed, with continually changing assumptions and a steady improvement in the experiments. Not surprisingly, conflicting values for the parameters have often been obtained, so that there is a need for a book such as this to assess the present position.

The chapter by Pisut on analytic extrapolation is of the most general implication for this type of work. Most analyses involve extrapolating a scattering amplitude, considered as a function of energy or some other complex variable, from a region where it is approximately known by experiment to some other point or arc, where it gives the value of a coupling constant, or the amplitude for another reaction. The stability of such extrapolations under small changes of experimental data has recently been considered in some intimidatingly mathematical papers. Pisut lucidly conveys the ideas behind these, and their conclusion that in certain cases only the average over an arc, not the value at a point, can be determined reliably. This chapter is closely tied in with one by Morgan and Pisut, which gives a clear and critical guide to the welter of analyses of pion-pion scattering.

The other major chapter is B. R. Martin's comprehensive review of kaon-nucleon interactions below the resonance region. This authoritatively covers the many phase-shift and K-matrix fits, and is particularly strong on the use of dispersion relations and sum rules. In related shorter articles A. D. Martin deals with determinations of the KN coupling, and Pilkun with SU(3) and PCAC predictions for baryon-meson couplings.

Pion-nucleon scattering is not covered, except for a discussion by Oades of Coulomb corrections. In nucleon-nucleon scattering, which has not changed much recently, analyses to find the couplings of exchanged mesons are reviewed by Kramer.

Photon-hadron interactions are represented by Gourdin, dealing with vector meson dominance, and Pfeil and Schwela on photoproduction of pions, etas and kaons.

Michael gives a good review of couplings from the viewpoint of Regge theory, vector dominance and SU(3). This chapter goes some way to remedy the chief weakness of the book, which is the lack of general discussion of the implications for currently fashionable ideas of all the constants so laboriously evaluated.

Appropriately, the book concludes with the useful, and steadily growing,

compilation of coupling constants and low energy parameters by Ebel and his colleagues.

The general layout is attractive, except for the typescript format of the final compilation. An index would have been a help, as the book will be used mostly for reference. But these are minor criticisms of a volume which many experimental and theoretical particle physicists will find of considerable value.

A. T. DAVIES

Ancient and Modern

Modern Methods in the History of Medicine. Edited by Edwin Clarke. Pp. xiv+389. (Athlone: London, April 1971. Distributed by Tiptree: Essex.) £5.50.

THIS is a salutary book, at a time when so many people are preaching how interesting and important medical history is, without, perhaps, sufficient attention to its academic quality. Dr Clarke has, naturally, had rather more than difficulty in keeping his collaborators to the matter in hand, but even when they pursue side issues they are well worth reading. The book makes rather a heavy start with two papers from America, where an academic style all too reminiscent of the turgidity of nineteenth century Germany is not unknown (how do you "create paradigmatic models which shape ideas of disease and disease causation"?). But later chapters from the United States restore the balance, and the reader is immediately refreshed by a splendid paper in English from Rhodesia. Professor McKeown gives an illuminating example both of strict thinking and of the demographic method of attacking the well known problem of the increase of population in the eighteenth century, and Professor Holmes of Yale provides a fascinating chapter on the use of "case histories" (stories of how single great discoveries were made) as examples of scientific method, and on the suggestion that the intellectual processes of advanced research differ from those required for making simple discoveries. Professor Fullmer's chapter on the history of biography as an art, and its different attitudes, objectives and interests at different times, is most illuminating.

The range of the book is one of its attractions. It covers much more than recent methods: it deals largely with new attitudes to the whole subject, away from the old fashioned seeking for "first descriptions" and priorities of discovery, through current interest in the history of ideas and of medical technologies, into new aspects of social medicine and such notions as the use of the study of what is happening in developing countries now, and throw-

ing light on corresponding developments in the past. Oral history, computers and other modern methods are included, but they are not really so interesting as the changing way in which the minds of medical historians are themselves working. New ways of looking at things are more significant than new technical tools; the whole book effloresces with original ways of looking at medical history.

There is one technical method about which a word must be said: the experimental method. It is often said—I have been guilty for one—that the scientist has the great advantage over the historian that he can not only observe but can experiment also, and here is Dr Clarke describing in a first class paper the way in which experiment can actually be applied to medical history, giving no less than thirty-five examples. Whatever the disabilities of ordinary historians, medical historians are more fortunately placed than some of them realize. The whole book is very encouraging. The discipline of medical history has come a long way in the past fifty years, but it is an exacting discipline. Its professionals are naturally sometimes exasperated by the efforts of amateurs, but this is the sort of book which will help to educate the “weekend” historians, and encourage the desirable union of amateur enthusiasm with professional carefulness.

CHARLES NEWMAN

Palaeopopulation

History of Human Life Span and Mortality. By Gy. Acsádi and J. Nemeskéri. Pp. 346. (Akadémiai Kiadó: Budapest, 1970.) £4.20.

PALAEODEMOGRAPHY, the study of the vital statistics of earlier populations, should have a long history. For well over a century, ancient human cemeteries in various parts of the world have been studied in detail by anthropologists and anatomists. All too often, however, information on age composition, life expectancy and so forth has been left out, with attention being given too much to craniometric detail. Because of this, it is no wonder that palaeodemography has taken so long to get off the ground.

Acsádi and Nemeskéri produce in their book a range of information which demonstrates beyond doubt that the demography of earlier man is now a well defined field of study with a substantial amount of data to refer to. The authors have actually produced the first textbook in palaeodemography, and in assessing its value I think it is important to remember the pioneering nature of the work. I think the authors were right to attempt this review now, even though data are accumulating quite fast

and the picture promises to be far more complete in another decade. Not only is this a valuable source book for human biologists, especially demographers wishing to give a temporal perspective to their discipline, but it provides further proof for the archaeologist of the variety of information to be derived from skeletal series which is highly relevant to a full reconstruction of past population history.

The book consists of eight chapters and a very ample bibliography. In terms of chapter contents, the work is divisible into three major parts. First, there is a consideration of the theoretical, methodological and general human biological questions relating to mortality and life span. Following this, the special problems and limitations of palaeodemographic work are discussed in detail. This includes a useful review of the literature in this field. There is then a very valuable assessment of the various factors which are used in the determination of sex and age in skeletal finds. This will certainly be of reference value to the forensic scientist as well as the archaeologist and human biologist. This chapter is followed by four others which consider mortality through different periods, from Pleistocene and Mesolithic populations to mediaeval times.

Perhaps naturally, examples tend to be from eastern Europe, where the authors have most knowledge. It was satisfying to find a discussion of the merits of age-estimations on fossil man. Ages, based on maturation rates in modern populations, have been attempted by some even on early Pleistocene hominids. Such procedures could well distort the true biological facts considerably, and it is important to be critical of them. Finally, the remaining section of the book gives a series of palaeodemographic life tables of various series referred to in the text. Cemetery samples are never as large as one desires, but Acsádi and Nemeskéri have certainly squeezed the maximum amount of data from these early series.

The book is lavishly produced, and unfortunately this is reflected in the relatively high price.

DON BROTHWELL

No Room for *Mus*

The Haemostatic Mechanism in Man and Other Animals. Edited by R. G. Macfarlane. (Symposia of the Zoological Society of London, No. 27.) Pp. xiv+248. (Academic: London and New York, November 1970. Published for the Zoological Society of London.) £4.50; \$13.50.

In December 1969 the Zoological Society of London arranged a two day

meeting at the Nuffield Institute of Comparative Medicine, to discuss the comparative physiology of haemostasis; this book, published within a year, offers 230 or so pages of its proceedings. The conference was a thoroughly successful innovation and is well worth repeating, because human physiologists cannot too frequently meet and debate with their zoological confrères.

The book starts, as did the meeting, with an urbane witty review by Professor R. G. Macfarlane, organizer of the conference, and ends with a general summary by Dr Christine Hawkey who, on her home ground in Regents Park, did so much to make the symposium successful.

For anyone—physiologist, pathologist or zoologist—this work succinctly reviews current knowledge of the haemostatic mechanisms in several species of vertebrates and invertebrates. Wherever feasible, changes are related to the parallel phenomena in man. Rosemary Biggs discusses human clotting mechanisms; A. E. Needham and C. Grégoire (Liège) cover respectively invertebrate lymphostasis and haemolymph coagulation; G. V. R. Born discusses platelet functional physiology; a team from Oxford—E. P. Adams, A. T. Nurden and John French, whose untimely death last year we lament—discuss platelet histochemistry, while D. C. B. Mills reviews platelet aggregation and nucleotide content in different species.

Haemostasis in the hamster (A. G. Sanders) and in fish, amphibia and other non-human vertebrates (R. K. Archer) takes the reader into a taxonomic maze from which he may be extricated by frequent reference to an abbreviated classification of the animal kingdom inserted into the front of the book. Christine Hawkey has admirably condensed her pioneer studies of fibrinolysis in a fascinating range of animals from the nose-horned viper to the skink, from the caiman to the cotton teal, and the cassowary and the argus pheasant to the vampire bat.

This is not a book into which one merely dips. Those even remotely interested in haematology should read it and assimilate K. W. E. Denson's review of abnormal clotting factors; B. Blombäck's (Sweden) and T. Cartwright's comments on evolutionary trends in the structure of fibrinogen, and N. O. Solum's (Norway) description of coagulation in *Limulus*, the horseshoe crab.

No fewer than 185 different species receive textual reference. What a pleasure to review work where *Rattus*, *Lepus* and *Canis* do not take pride of place, and *Mus* is not even mentioned.

J. L. STAFFORD

CORRESPONDENCE

Scientific Salaries

SIR,—Your editorial comment on the dispute on the pay of scientists in government service (*Nature* 232, 76; 1971) would have been more informative had it stated clearly that the pay claim on behalf of the scientists asked for no more than parity with other comparable classes within the service, all of whom have had increases this year. Despite the demanding and creative work and extra academic qualifications of many officers in scientific grades, their pay until recently has been tied to that of the administrative grades. But, as one result of the present pay research exercise, Principal Scientific Officers (an important body of scientists among those that were offered no increase and now paid in the course of nine annual increments from between £2,820 and £3,902 a year) were henceforth to receive between £430 and £749 less than their colleagues in administration. Hence some of the anger to which you refer.

The result is to demote science relative to other professions. Quite apart from our personal dissatisfactions, we are apprehensive lest minds of high calibre at schools and in universities are discouraged from following a scientific career. Science, technology and the country will suffer.

Yours faithfully,

NIGEL BATEMAN

*Institute of Professional Civil Servants,
Animal Breeding Research Organization,
Edinburgh EH9 3JQ*

BNF Subscriptions

SIR,—Some comment seems necessary on your remarks in the report on the discussion by the House of Commons Select Committee on Science and Technology of the work of the Department of Trade and Industry (*Nature* 231, 208; 1971). You refer to the deficit for 1970 of £36,618 in the accounts of the British Non-Ferrous Metals Research Association and the decision of our Council to raise subscriptions by 25%. This deficit should be viewed in the context of a total income for the year of almost £700,000. The short-fall in a period of rapidly rising costs was about 5% of the budget and follows a series of modest surpluses.

The decision to raise subscriptions was taken by our Council several months ago and was a reflexion of the increased costs of providing services to members since the last general increase in subscription rates in 1967. There must be few concerns

successful in resisting price increases over such a long period. It has been possible at the BNF partly because of the continuing success in recruiting members from overseas (they now account for one-third of membership subscriptions) and partly through a policy of accepting more contract work. Contract income, including contracts for groups of members, reached £370,000 in 1970.

Yours faithfully,

E. C. MANTLE

*Deputy Director,
The British Non-Ferrous Metals
Research Association,
Euston Street, London, NW1*

Acknowledgments

SIR,—Your article (*Nature*, 232, 75; 1971) was in my opinion priceless but the subject does pose some important questions, especially when costs of publications are rising so rapidly and of necessity economies have to be made.

Of course the editors of *Nature* have the power to demand the elimination of all fancy "acknowledgments"—if no agreement then no publication. In my experience from both sides of the fence, most authors will suffer almost any indignity as long as their contributions are accepted for publication. The omission of the names Rosemary Smith and Fred Brown therefore would not cause undue alarm; rather such an editor's admonition provides an adequate excuse under the circumstances. "Sorry folks but the editor insists; you know what the blighters are like, etc., etc.". Nevertheless, what does one do with a grantbody which lays down that any publication must include an acknowledgment of the source of any financial support. Again some heads of departments (so I'm told) probably expect at the very least a mention, even for their hospitality. Sometimes they may even deserve it too. Such reference is probably a lesser evil or less hypocritical than including superfluous names on the title page.

Perhaps, however, common sense and compromise can at least prevail. When papers are particularly long then "acknowledgments" of grander proportions, if genuine, would seem to be justified. With short papers, found for example in *Nature*, brevity would seem to be in order.

Finally, a plea for the technician. Many do assist wholeheartedly and very substantially in research projects; indeed as scientific research becomes more

complex and techniques more specialized, highly trained technical experts are now becoming indispensable. Though most technicians presumably do not participate in writing research papers, in fact many individuals doubtless hardly understand parts of them, and even though everyone knows that they get paid, the very least authors can do is politely acknowledge important contributions by them. The cost in relative terms is small but even in this day and age appreciation of one's colleagues' efforts should still not be amiss.

Yours faithfully,

HAROLD FOX

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Neolithic *Spondylus*

SIR,—I should like to refer to the interesting article by Shackleton and Renfrew¹ on the use of the 18/160 technique with shells of *Spondylus gaederopus* L. in the investigation of Neolithic trade routes.

As far as the genus *Spondylus* L. is concerned, it is a typical tropical form, one of the representatives of which, *S. gaederopus*, is known to inhabit Mediterranean waters as well. Its history in the different Mediterranean basins shows that while in the West² it is known from as far back as the Miocene and the Pliocene, we do not find it in the eastern basins before the Tyrrhenian Period, where it is recovered in various outcrops in Cyprus³, the Dodecanese Islands and so on.

S. gaederopus is not found today in the Black Sea, owing to the special properties of this water mass, which are mentioned by Shackleton and Renfrew, and are quite different from those of the Mediterranean. According to the authors themselves, the isotopic composition of the Black Sea and the Mediterranean waters was even more different in the past than today, because of the greater influence of the glacial meltwater. If so, the probability of finding such a tropical to sub-tropical organism in the Black Sea, from its last opening and connexion with the Mediterranean some 8,000 to 10,000 years ago⁴, is even more unexpected. On the other hand, it is possible that during another warm period with a high sea level in the Tyrrhenian (some 115,000 to 140,000 years ago)⁵ when even *Strombus bubonius* Lmk. intruded

into the Black Sea⁶, we might expect to find *S. gaederopus* also. However, according to Shackleton and Renfrew¹, the radiometric age of the examined *Spondylus* shells is only 2,500 BC and 4,000 BC.

It is therefore concluded that, at least for this special case, the ^{18/16}O method is irrelevant. Finally, *S. gaederopus* lives today along various Mediterranean coasts (Algeria, France, Italy, Israel, and so on) cemented to

some rocky substrate, and it would probably be very difficult to tell from the isotopic composition the exact geographical location (that is Aegean or Adriatic) where the samples were collected.

Yours faithfully,

S. MOSHKOVITZ

Department of Geology,
Hebrew University of Jerusalem

Announcements

University News

Professor P. M. Maitlis, McMaster University, has been appointed to the chair of inorganic chemistry, University of Sheffield.

Appointments

Mr W. A. Cumming and Dr J. D. Keys have each been appointed assistant vice-president (laboratories) of the National Research Council of Canada.

Graham Morris, manager of the Education and Training Division of ICL, has been appointed deputy president of the British Computer Society for 1971-72.

Sir Ronald Baskett has been appointed secretary of the Agricultural Research Council, in succession to Sir Gordon Cox.

Mr D. J. Parkinson has been appointed under secretary, and Dr C. C. Webster has been appointed chief scientific officer.

Miscellaneous

The Academy of Sciences of the USSR has been awarded the International Galaber Prize for the research projects carried out by the automatic spacecraft Luna-16.

Dr F. B. A. Giwa, University of Ibadan, has been awarded the first World Meteorological Organization Research Award for Regional Association I (Africa), in recognition of his work on the effect of wind on the resonant period of the atmosphere.

logical Organization Research Award for Regional Association I (Africa), in recognition of his work on the effect of wind on the resonant period of the atmosphere.

Erratum

In the article "Australia's Caenozoic Drift" by J. G. Jones (*Nature*, 230, 237; 1971), the first sentence of the penultimate paragraph should read "A final problem which Australia's Caenozoic drift may elucidate is the origin of the Coral Sea".

British Diary

517th Meeting (two days) Biochemical Society, in the Appleton Tower, University of Edinburgh, George Square, Edinburgh. Biological Hydroxylation Mechanisms (symposium); and meeting for the presentation of free communications.

Reports and Publications

not included in the monthly Books Supplement

Other Countries

Carte Pédologique de la France au 1/100,000. Argeles-sur-Mer—Perpignan, L. 24 et L. 25. Notice explicative par J. Servant. Pp. 114. (Versailles: Institut National de la Recherche Agronomique, 1970.) 53.75 francs. [274]
Nederlandse Vereniging voor Weer- en Sterrenkunde. Observations of Variable Stars, July-December 1970. (Report No. 19.) Pp. 7. (Groningen, Netherlands: Kapteyn Astronomical Laboratory, 1971.) [274]

- ¹ Shackleton, N., and Renfrew, C., *Nature*, 228, 1062 (1970).
- ² Bucquoy, E., Dautzenberg, Ph., and Dollfus, G. (B.D.D.), *Les Mollusques marins de Roussillon* (1882-1898).
- ³ Moshkovitz, S., in Bear, L. M., *Palaeontological Notes, Ann. Rep. for the Year 1962* (Geol. Surv. Dept., Cyprus).
- ⁴ Bukry, D., King, S., Horn, M. K., and Manheim, F. T., *Nature*, 226, 156 (1970).
- ⁵ Butzer, K. W., *Encyclopedia of Oceanography* (edit. by Fairbridge) (Reinhold Publ. Corp., 1966).
- ⁶ Caspers, H., *Geol. Soc. Amer. Mem.* 67, 1, 801 (1957).

CERN—European Organization for Nuclear Research. CERN 71-8: Spin Formalisms. By S. U. Chung. (Lectures given in the Academic Training Programme of CERN 1969-1970. Pp. v+81. (Geneva: CERN, 1971.) [274]

National Academy of Sciences; National Academy of Engineering; National Research Council. Organization and Members 1970/1971. Pp. 243. (Washington, DC: National Academy of Sciences; National Academy of Engineering, 1970.) [284]

NINDS Research Profiles: 1970—Summary of Research at the National Institute of Neurological Diseases and Stroke. (US Department of Health, Education and Welfare. Public Health Service, National Institutes of Health.) Pp. vii+57. (Washington, DC: Government Printing Office, 1970.) \$0.35. [284]
Health Sciences in Israel—Institutions and Scientists. Edited by Betty Davies. Pp. x+285. (Jerusalem: Israel Journal of Medical Sciences, 1971.) \$7. [284]

US Department of the Interior: Geological Survey. Professional Paper 537-E: Chronological Narrative of the 1959/1960 Eruption of Kilauea Volcano, Hawaii. By D. H. Richter, J. P. Eaton, K. J. Murata, W. U. Ault, and H. L. Krivoy. Pp. vi+73. Water-Supply Paper 1878: Water Resources of Racine and Kenosha Counties, South-eastern Wisconsin. By Rickard D. Hutchinson. Pp. v+63+4 plates. (Washington, DC: Government Printing Office, 1970.) [284]

US Department of the Interior: Geological Survey. Water-Supply Paper 1879-C: Ground-Water Aspects of the Lower Henrys Fork Region, Eastern Idaho. By E. G. Crosthwaite, M. J. Mundorff and E. H. Walker. Pp. iii+22+1 plate. \$0.70. Water-Supply Paper 1895-B: Summary of Data on Chemical Quality of Streams of North Carolina, 1943-67. By Hugh B. Wilder and Larry J. Slack. Pp. iv+236+2 plates. (Washington, DC: Government Printing Office, 1970 and 1971.) [294]

US Department of the Interior: Fish and Wildlife Service. Bureau of Sport, Fisheries and Wildlife. National Wildlife Refuge. Pp. 28. \$0.35. Wildlife Research: Problems, Programs, Progress, 1968. Pp. viii+112. \$1.25. (Washington, DC: Government Printing Office, 1970.) [294]

Research Council of Alberta. Annual Report 1970. Pp. 75. (Edmonton: Research Council of Alberta, 1971.) [294]

Smithsonian Contributions to Paleobiology. No. 3: Paleozoic Perspectives—a Paleontological Tribute to G. Arthur Cooper. Edited by Thomas Durrell, Jr. Pp. xiv+390. \$4. Smithsonian Contributions to Zoology. No. 69: Biostatistical Programs in BASIC Language for Time-Shared Computers. Coordinated with the book "Quantitative Zoology". By James A. Peters. Pp. 46. \$0.50. No. 77: Bredin-Archbold-Smithsonian Biological Survey of Dominica—Bourrowing Sponges, Genus *Siphonodictyon* Bergquist, from the Caribbean. By K. Rützler. Pp. 37. \$0.50. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [294]

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